Optimising nitrogen fertilisation of kikuyu and kikuyu-ryegrass pastures

by
Charné Viljoen

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Supervisor: Dr Pieter Swanepoel
Co-supervisor: Ms Janke van der Colf

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Declaration

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Summary

Dairy production in the southern Cape is mostly based on irrigated planted pastures. Pure grass pastures [kikuyu (*Pennisetum clandestinum*) and ryegrass (*Lolium* spp.)] are often established by using minimum-tillage methods. One of the most important management practices of kikuyu-ryegrass pastures is nitrogen (N) fertilisation. The current N fertilisation guidelines often recommend more than 500 kg N ha^{-1} yr^{-1}, which is possibly too high. The guidelines need to be re-evaluated, since it was developed under cutting conditions using conventional-tillage, and may not be accurate for the minimum-tilled and grazed systems. The aim of this study was to determine an optimum rate of N application of kikuyu and kikuyu-ryegrass pastures, either by a fixed N fertilisation rate or a variable rate according to the demand of the plant in a specific season. Six N fertilisation treatments, one variable rate (Nvar) and five fixed rates (0, 20, 40, 60 and 80 kg N ha^{-1} grazing $cycle^{-1}$) were used in the current study. Treatment Nvar was based on the soil water nitrate concentration obtained from using wetting front detectors (WFD). The nitrate concentrations and total soil mineral N indicated that a major pool of N is vulnerable to potential leaching losses. In both kikuyu and kikuyu-ryegrass systems, applications above 40 kg N ha^{-1} grazing $cycle^{-1}$ indicated a build-up of total mineral N in soil. No difference between the lower N treatments (≤ 40 kg N ha^{-1}) was found in terms of total mineral N. Mineral N and urease enzyme activity were the only soil parameters that were affected by treatments. Urease activity of the control treatment (no N) was mostly higher ($P \le 0.05$) compared to the 80 kg N ha^{-1} treatment. Total soil N resulted in seasonal differences and was considered to be related to variation in seasonal herbage production. For example, during periods of high pasture production total soil N in soil was low, but increased during periods of low pasture production. For both kikuyu and kikuyu-ryegrass pastures, the highest herbage production was during spring and summer, while the lowest total soil N was found during summer and autumn. On the kikuyu site, N treatments had an effect on the herbage production during all the seasons of year one, but not during year two. On the kikuyu-ryegrass site, N treatment affected the production during winter, spring and summer of year one, and during the summer of year two. As N treatments increased on both the study sites, the self-sown clover component decreased. Agronomic N use efficiency was similar across treatments and seasons on the kikuyu and kikuyu-ryegrass site, with the exception of winter in the first year in the kikuyu-ryegrass site. This supports the notion that the soil is saturated with N. Crude protein (CP) content of herbage increased with an increase in N, to a point where CP was too high for milk production for some treatments. It is concluded that the current N guidelines needs to be revisited as they pose a risk to the environment and farm economics.

Opsomming

Suiwelproduksie in die Suid-Kaap is meestal op aangeplante weidings onder besproeiing. Suiwer grasweidings [kikoejoe (*Pennisetum clandestinum*) en raaigrass (*Lolium* spp.)] word deur minimum-bewerkingsmetodes gevestig. Een van die belangrikste bestuurspraktyke van kikoejoe-raaigras weidings is stikstof-(N)-bemesting. Die huidige N-bemestingsriglyne beveel dikwels N-peile van meer as 500 kg N ha^{-1} $jaar^{-1}$ aan, wat moontlik te veel is. Hierdie riglyne behoort weer ondersoek te word, aangesien dit op snyproewe en konvensionele bewerkingstelsels ontwikkel is. Die doel van hierdie studie was om 'n optimale N-peil vir kikoejoe- en kikoejoe-raaigrasweidings te bepaal, hetsy deur vaste N-bemestingspeile of 'n veranderlike peil volgens die behoefte van die weiding in 'n spesifieke seisoen. Ses N-bemestingsbehandelinge, een veranderlike peil (Nvar) en vyf vaste peile (0, 20, 40, 60 en 80 kg N ha^{-1} $weisiklus^{-1}$) was in die huidige studie gebruik. Die behandeling Nvar, was op nitraatkonsentrasies van die grond gebaseer, wat deur benattingsfrontaanwysers bepaal is. Die nitraatkonsentrasies en die totale minerale N van die grond, het 'n groot poel potensiële loogbare N aangedui. In beide kikoejoe- en kikoejoe-raaigrasstelsels het N-toedienings bo 40 kg N ha^{-1} $weisiklus^{-1}$, 'n opbou van totale minerale N aangedui. Daar was geen verskille tussen die laer N-behandelings (≤ N40) nie. Minerale N en urease-aktiwiteit was die enigste grondparameters wat deur N-behandeling beïnvloed is. Urease-aktiwiteit van die kontrole (geen N) was meestal hoër in vergelyking met die van 80 kg N ha^{-1} $weisiklus^{-1}$. Totale grond N het seisoenale verskille tot gevolg gehad en dit word vermoed dat dit met seisoenale weidingsproduksie verband hou. Byvoorbeeld, gedurende periodes van hoë weidingproduksie was totale grond N laag, terwyl dit gestyg het gedurende periodes van lae weidingproduksie. Op beide kikoejoe- en kikoejoe-raaigrasstelsels was die hoogste weidingsproduksie gedurende lente en somer, terwyl die laagste totale grond N gedurende somer en herfs gevind is. Op die kikoejoe-weiding het N behandelinge 'n effek op die weidingsproduksie gedurende al die seisoene van jaar een, maar nie gedurende die tweede jaar gehad nie. Op die kikoejoe-raaigrasweiding het N-behandelinge die produksie gedurende winter, lente en somer van jaar een, en gedurende die somer van die tweede jaar beïnvloed. Op beide weidings, namate die N-behandelinge toegeneem het, was daar 'n afname in die klawerbydrae. Agronomiese N-doeltreffendheid was soortgelyk oor behandelings en seisoene op die kikoejoe- en kikoejoe-raaigrasweidings, met die uitsondering van winter in die eerste jaar op die kikoejoe-raaigrasweidings. Hierdie versterk die stelling dat die grond N versadig is. Die inhoud van ru-proteïen (CP) van albei weidings het toegeneem met 'n toename in N-behandeling tot 'n punt waar CP te hoog geraak het vir melkproduksie in sommige behandelings. Die gevolgtrekking word daarom gemaak dat die huidige N riglyne heroorweeg moet word aangesien dit waarskynlik tot omgewings- en finansiële verliese sal lei.

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Abbreviations

ANOVA	analysis of variance
ANUE	agronomic nitrogen use efficiency
B	boron
°C	degrees Celsius
C	carbon
c.	circa (about)
Ca	calcium
$CaCl_2$	calcium chloride
cm	centimeter
CP	crude protein
Cu	copper
cv.	cultivar
DM	dry matter
dS	deciSiemens
et al.	and others
g	gram
h	hour
ha	hectare
K	potassium
KCl	potassium chloride
Kg	kilogram
kPa	kilo Pascal
L	litre
LAN	limestone-ammonium nitrate

Mg	magnesium
mL	millilitre
mm	millimetre
mM	millimole
Mn	manganese
MPa	megapascal
N	nitrogen
Na	sodium
NaCl	sodium chloride
NDF	neutral detergent fibre
NMDS	non-metric multidimensional scaling
P	phosphorous
pH	value that specifies solution as acidic (<7) or basic (>7) based on hydrogen ions
PMN	potentially mineralisable nitrogen
REML	restricted maximum likelihood
S	sulphur
spp.	species
t	ton
µ	micro
TNC	total non-structural carbohydrates
viz.	videlicet (namely)
vs.	versus (in contrast to)
WFD	wetting front detector
Yr	year
Zn	zink

Chapter 1 Introduction

1.1 Background

Kikuyu (*Pennisetum clandestinum*), a C_4-grass, forms the base of pastures (Marais 2001; Garcia et al. 2014) in various areas in the world, including Australia (Fulkerson et al. 1999; Bhanugopan et al. 2010), New Zealand (Crush and Rowarth 2007) and the Americas (Williams and Baruch 2000). Kikuyu is also used as a pasture base for grazing dairy cows in the southern Cape region of South Africa (Botha et al. 2008a). The production systems and climate in the southern Cape area favour the growth of kikuyu. Irrigation infrastructure is usually available if rainfall is insufficient (Botha 2009), the area has a temperate climate (Marais 2001; Swanepoel et al. 2014a) and pastures are most often fertilised with inorganic nitrogen (N) (Garcia et al. 2014). The interaction of these factors from year to year results in variation in the production potential. Within the same experimental site, kikuyu production has been recorded from as low 14 t dry matter (DM) ha^{-1} yr^{-1} (Botha et al. 2008a) to as high as 21 t DM ha^{-1} yr^{-1} (Swanepoel et al. 2014b).

Kikuyu offers a resilient pasture base due to its growth form which is comprised of above ground stolons and below ground rhizomes, thus withstanding damage from trampling from grazing animals (Garcia et al. 2014). For this reason, past research failed to provide a cost-effective way to eradicate kikuyu, and therefore later focussed instead on ways to incorporate kikuyu into systems as a pasture base (Botha 2009). Eradication of kikuyu was historically considered as it has undesired characteristics which include, among others, dormancy in winter and spring, resulting in a deficit in the fodder flow program during these months (Lowe et al. 2011). Furthermore, kikuyu, are usually not of adequate quality for high producing dairy cows (Marais 2001; Fulkerson 2007; Fulkerson and Lowe 2011). Kikuyu's low quality and mineral imbalances (Reeves et al. 1996; Marais 2001) could be overcome by supplementing dairy cows with high energy concentrates such as cereal grains (Fulkerson 2007). This is confirmed by Meeske et al. (2006), who found that feeding low or medium levels of concentrates (consisting of amongst others whole cottonseed, rolled maize and rolled wheat), increased the fat-corrected milk production compared to those receiving no concentrates. The cows in the study grazed pasture which consisted of 15% kikuyu. However, strategic incorporation of other grasses into kikuyu has been found to be an economical forage-based way to improve the pasture productivity, in addition to supplementing cows with concentrates (Botha et al. 2008a; Sinclair and Beale 2010; van der Colf 2011). The aggressive and dominant growth capacity of kikuyu limits the persistence of some species sown into the kikuyu-base, particularly clovers and other legumes (Sinclair and Beale 2010; Garcia et al. 2014). However, ryegrass (*Lolium* spp.), a genus of cool-season grasses, is often sown into kikuyu to compliment the dormant kikuyu pastures during winter and spring in terms of herbage production (Bell et al. 2011; Garcia et al. 2014) and overall pasture quality (Reeves et al. 1996).

Common practice is to graze the kikuyu base down to 50 mm above ground level, after which it will be mulched to ground level and planted with ryegrass using minimum-tillage seed-drills. A land roller is then used to roll the seedbed, followed by irrigation. This method is explained in detail by Botha (2009) and van der Colf (2011) and confirmed to be a good option, compared with chemical or cultivation methods, to avoid production losses (Swanepoel et al. 2014b). Such kikuyu-ryegrass systems can maintain herbage production rates between 20.8 and 23.9 t DM ha^{-1} yr^{-1} as found in Australia (Fariña et al. 2011). Locally, in the southern Cape of South Africa, previous studies found herbage production to be in the range of 13.5 to 20.3 t DM ha^{-1} yr^{-1} (Botha et al. 2008; Swanepoel et al. 2014b; van der Colf 2015)

Nitrogen fertilisation guidelines for kikuyu-ryegrass pastures recommend application rates between 300 and 500 kg N ha^{-1} yr^{-1} (Marais 2001). When kikuyu is heavily fertilised with N, it can result in crude protein (CP) contents that exceed the requirement of the dairy cow (Marais 2001; Fessehazion et al. 2011). The CP requirement for lactating dairy cow is on average 16% (Clark et al. 2001; NRC 2001), but can also be between 17% and 19% (Radostits et al. 2006).

In the Eastern Cape of South Africa, non-protein N (mostly in the form of nitrate) varied considerably in kikuyu and was found to be between 0.43 and 14.33% (Miles et al. 2000). Management of kikuyu pastures should include strategies aimed at preventing the accumulation of large amounts of stem material since nitrate tends to accumulate in the stem material (Marais 2001). Nitrate levels that could be toxic to animals, may exist when large amounts of N fertiliser is applied (Adams et al. 1992; Ju et al. 2004) due to kikuyu’s tendency to accumulate non-protein, nitrogenous compounds (Marais 2001). In general, animal nitrate poisoning may be prevented by taking caution when and where grazing of pasture takes place. Bolan and Kemp (2003) reviewed factors causing nitrate accumulation in pasture which included growth stage (early growth or mature leaves and stems), drought, limited sunlight and plant stress factors (e.g. herbicide or disease damage). These factors hinder the plant’s ability to transform nitrate to ammonium to organic compounds (proteins). The digestion of non-structural carbohydrates may be reduced when nitrate levels above 5 g kg^{-1} are present in the plant (Marais et al. 1990) and it is therefore recommended that less than 0.6% nitrate is ingested in the diet (Radostits et al. 2006).

Grazing management of these kikuyu-ryegrass systems is well-researched and is used in practice (Fariña et al. 2011). The grazing goals for temperate grass pastures are to maximise yield, while in tropical pastures the main aim is to improve forage quality (Fulkerson and Lowe 2011). To determine when grazing should commence, leaf stage could be used. An optimal nutritive value is achieved at a 4.5 leaf stage for kikuyu (Reeves et al. 1996; Marais 2001). Grazing of ryegrass should commence at the three-leaf stage to prevent wastage of herbage and a reduction of forage quality (Reeves et al. 1996; Fulkerson and Donaghy 2001). This, however, is not the only way of determining the grazing interval for pasture. Other methods include set days (Fulkerson and Lowe

2011), pasture height measured with pasture ruler (Sanderson et al. 2001) and DM-on-offer using a rising plate meter (Thomson et al. 2001).

If it is possible to supply a sufficient amount of good quality pasture, less concentrate is needed. It was shown that the highest margin over feed cost was obtained when low amounts of dairy concentrate were fed (3 kg cow^{-1} day^{-1}) to Jersey cows, also allowing 20 kg of pasture cow^{-1} day^{-1} (Meeske et al. 2006).

For any farming operation to be successful, it needs to be profitable. The aim of a dairy farm is to produce milk at a profit and thus produce it at the lowest cost or greatest efficiency. As N fertilisation is one of the highest input costs, it needs to be optimised (Aarts 2000).

1.2 Problem statement

Nitrogen, in the form of nitrate, is soluble in water and therefore the roots are able to use it through water uptake via the plant's roots (Good et al. 2004). When the plant is not actively growing (i.e. dormant) and too much N is applied, excess N easily leaches into the groundwater (Varvel et al. 1997; Errebhi et al. 1998; Di and Cameron 2002). Irrigated pasture systems used for dairy production are seen as one of the agricultural systems with the largest amounts of N fertiliser inputs (Baligar et al. 2001; Masclaux-Daubresse et al. 2010; Fessehazion et al. 2011). In order to maximise pasture yield, kikuyu-ryegrass pastures are usually fertilised with high rates of N, and are therefore more vulnerable to N losses. Aside from the detrimental effects leached N that may have on the environment, such as eutrophication (Nixon 1995; Conley et al. 2009), N is an expensive input. When N is lost to the environment, it is a major financial loss to farmers. Even though denitrification and volatilisation are also pathways through which N could be lost, these comprise a smaller portion compared to losses attributed to leaching (Ledgard et al. 1999).

Nitrogen guidelines in the southern Cape should be revisited in order to prevent losses, especially through leaching. The current kikuyu-ryegrass pasture fertilisation guidelines are based on cultivated pastures. Since minimum-tillage systems are currently used more often than conventional tillage systems (Swanepoel et al. 2015) these guidelines may no longer be applicable. The conversion from conventional tillage to minimum- or zero-tillage systems generally result in a decreased risk of soil erosion (Swanepoel et al. 2013), less N leaching (Di and Cameron 2002), better water holding capacity, higher soil organic carbon (C) (López-Garrido et al. 2011) and increased potential mineralisable N (PMN) (Balesdent et al. 2000; Swanepoel et al. 2014c). Furthermore, the current N guidelines were also developed based on data obtained from cutting trials (Beyers 1994), and as a result, does not take into account the animal input through excreta. Bransby (1990) suggested that the reaction of pasture to N fertilisation might not be comparable between pasture grazed by animals and pastures being mowed (cutting trials), because the recycling of nutrients is different. This is demonstrated by the vast amount of N leaching studies of urine patches (Thompson and Fillery 1998; Di and Cameron 2000; Cameron and Di 2004; Christensen et al. 2010; Roten et al. 2017) where it is estimated that the localised application rate on urine patches may reach up to 1000 kg N ha^{-1} (Haynes and Williams 1993). Mineral N losses increased by 14% when N fertiliser as urea was applied over urine patches (Silva et al. 1999).

The challenge is to optimise farming efficiency by reducing N losses and thereby indirectly reducing input costs to increase profitability while being environmentally sustainable. Various authors have suggested incorporating legumes, like clover (*Trifolium* spp.), into the pasture to reduce N fertiliser input. However, this runs the risk of reducing the pasture production compared to pure grass stands (Peoples and Baldock 2001; Andrews et al. 2007; Ledgard et al. 2009). Drawbacks of clover inclusion in a system include the sub-optimal establishment (Brock and Kane 2003; Schlueter and Tracy 2011) and persistence-problems (Caradus and Woodfield 1995) resulting in lower herbage production and, in turn, lower stocking rates.

The higher production and stocking rates of pure grass pastures are, however, often accompanied by high rates (>500 kg N ha^{-1} yr^{-1}) of N fertilisation and therefore an optimal N application rate should be determined to prevent financial and environmental losses. In determining the optimal N application rate of grazed dairy pastures, pasture and soil characteristics, as well as input from grazing animals, should be considered. Nitrogen fertilisation of kikuyu over-sown with temperate pasture species, should be managed in such a way that all the pasture components remain highly productive, without being compromised by excessive amounts of N fertiliser that may reduce the quality (Fessehazion et al. 2011). One strategy that has been recommended is strategic N applications based on applying various rates of N during different seasons according to pasture growth, soil supply of N and needs of the plant. Also, applying N in amounts where N use efficiency is high may reduce losses (Eckard and Franks 1998). There is abundant research on N fertilisation of clover and ryegrass pastures (Harris et al. 1996; Lowe et al. 2005; Labuschagne et al. 2006; Bolland and Guthridge 2007; Eckard et al. 2007; Gilliland et al. 2010; Pembleton et al. 2013), but research on strategic application of N on kikuyu-ryegrass systems is limited. On Cedara in KwaZulu Natal, Fessehazion et al. (2011) is one of the few studies which concluded that adaptive management on kikuyu-ryegrass pasture systems did not affect the herbage yield. Wetting front detectors (WFD) was used as a management practice to reduce the N input and thereby lowering the potential of N leaching. Research that focus on determining optimum N fertilisation rates for kikuyu-based pastures over-sown by ryegrass using minimum-tillage practices in the southern Cape, is currently lacking.

1.3 Aim

To determine an optimum rate of N application of kikuyu and kikuyu-ryegrass pastures either by a fixed N fertilisation rate or a variable rate according to the demand of the plant in a specific season.

1.4 Objectives

1. The objective was to investigate the effects of fertilisation on soil N dynamics, which will aid in optimising N fertilisation of kikuyu and kikuyu-ryegrass pastures. In order to achieve the aim of the study, the effects of different N rates on various soil characteristics such as PMN, C:N ratio, urease activity (UA) and total soil N are determined in order to better understand the N dynamics in the soil as influenced by grazing, N fertiliser input and season.
2. The objective was to reassess N fertiliser guidelines of kikuyu and kikuyu-ryegrass pastures in the southern Cape under a minimum-tillage regime and grazing, aimed at optimising the quality of pasture, measured by CP, while still maintaining yield. Developing a strategic N fertiliser programme may aid in preventing environmental and financial losses since agronomic N use efficiency (ANUE) is taken into account.

1.5 Hypotheses

1. Adjusting the N fertilisation rates according to soil N characteristics will result in a better understanding of N dynamics in the soil and the development of strategies to prevent losses.
2. Lowering the current N guidelines will result in a reduction in financial and environmental N losses by increasing the ANUE of pasture and increase pasture quality as measured by CP.

1.6 Outline of the thesis

This thesis is presented in article-based format with Chapters 3 and 4. It is prepared according to the guidelines of the *African Journal of Range and Forage Science*. This introduction serves the purpose of tying Chapters 3 and 4 into a cohesive piece of work that explains the overall problem and scientific contribution. Subsequent to this introductory chapter (Chapter 1), a literature review (Chapter 2) was conducted in order to present an overview of the N cycle and the processes taking place in the soil. Furthermore, a broad overview of both pasture species used in the current study, kikuyu and ryegrass, is presented in Chapter 2, with emphasis on how N fertilisation affect these species.

Chapter 3 reports the effects that N fertilisation had on chemical and biological soil characteristics. These characteristics included total mineral N, PMN, soil water nitrate concentration, total soil N, soil carbon, and urease activity.

Chapter 4 is set out to determine the effects of N fertilisation on herbage production, botanical composition, agronomic N use efficiency and the quality of the pasture (by using CP).

Chapter 5 presents the conclusions, limitations of the study and recommendations for future research.

1.7 References

Aarts H. 2000. Resource management in a 'De Marke' dairy farming system. PhD thesis, Wageningen Universiteit, The Netherlands.

Adams RS, McCarty TR, Hutchinson LJ. 1992. Prevention and control of nitrate toxicity in cattle. *Penn State College of Agricultural Sciences Cooperative Extension Department of Animal Science* 92–107: 1-12.

Andrews M, Scholefield D, Abberton MT, Mckenzie BA, Hodge S, Raven JA. 2007. Use of white clover as an alternative to nitrogen fertiliser for dairy pastures in nitrate vulnerable zones in the UK : productivity, environmental impact and economic considerations. *Annals of Applied Biology* 151: 11–23.

Balesdent J, Chenu C, Balabane M. 2000. Relationship of soil organic matter dynamics to physical protection and tillage. *Soil and Tillage Research* 53: 215–230.

Baligar VC, Fageria NK, He ZL. 2001. Nutrient Use Efficiency in Plants. *Communications in Soil Science and Plant Analysis* 32: 921–950.

Bell MJ, Cullen BR, Eckard RJ. 2011. The production of perennial ryegrass and kikuyu pastures in south-eastern Australia under warmer and drier future climate scenarios. In: *19th International Congress on Modelling and Simulation, 12 – 16 December, Perth, Australia.* pp 753–759.

Beyers C de L. 1994. Die bemesting van aangeplante weidings. *Weidings = Pastures Elsenburg: Department of Agriculture*: 86–93.

Bhanugopan MS, Fulkerson WJ, Fraser DR, Hyde M, Mcneill DM. 2010. Carryover effects of potassium supplementation on calcium homeostasis in dairy cows at parturition. *Journal of Dairy Science* 93: 2119–2129.

Bolan NS, Kemp PD. 2003. A review of factors affecting and prevention of pasture-induced nitrate toxicity in grazing animals. In: *Proceedings of the New Zealand Grassland Association 65:* 171–178.

Bolland MDA, Guthridge IF. 2007. Responses of intensively grazed dairy pastures to applications of fertiliser nitrogen in south-western Australia. *Australian Journal of Experimental Agriculture* 47: 927–941.

Botha PR. 2009. Factors influencing the persistence and production potential of kikuyu (*Pennisetum clandestinum*) over-sown with different ryegrass and clover species in the southern Cape. *Elsenburg Joernaal* 3: 4–9.

Botha PR, Meeske R, Snyman HA. 2008a. Kikuyu over-sown with ryegrass and clover: grazing capacity, milk production and milk composition. *African Journal of Range and Forage Science* 25: 103–110.

Botha PR, Meeske R, Snyman HA. 2008b. Kikuyu over-sown with ryegrass and clover: dry matter production, botanical composition and nutritional value. *African Journal of Range and Forage Science* 25: 93–101.

Bransby DI. 1990. Nitrogen fertilization, stocking rate and rotational grazing effects on steers grazing *Pennisetum clandestinum*. *Journal of the Grassland Society of Southern Africa* 7: 261–264.

Brock JL, Kane GJ. 2003. Variability in establishing white clover in pastures on farms. In: *Proceedings of the New Zealand Grassland Association* 65: 223–228.

Cameron KC, Di HJ. 2004. Nitrogen leaching losses from different forms and rates of farm effluent applied to a Templeton soil in Canterbury, New Zealand. *New Zealand Journal of Agricultural Research* 47: 429–437.

Caradus JR, Woodfield D. 1995. Overview and vision for white clover white clover: New Zealand's competitive edge. *Special Publication-Agronomy Society of New Zealand* 11: 1–6.

Christensen CL, Hanly JA, Hedley MJ, Horne DJ. 2010. Reducing nitrate leaching losses by using duration-controlled grazing of dairy cows. *19th World Congress of Soil Science, Soil Solutions for a Changing World, Sydney, Australia* 153–156.

Clark DA, Matthew C, Crush JR. 2001. More feed for New Zealand dairy systems. In: *Proceedings of the New Zealand Grassland Association* 63: 283–288.

Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE. 2009. Controlling Eutrophication: Nitrogen and Phosphorus. *Science* 323: 1014–1015.

Crush JR, Rowarth JS. 2007. The role of C 4 grasses in New Zealand pastoral systems. *New Zealand Journal of Agricultural Research* 50: 125–137.

Di HJ, Cameron KC. 2000. Calculating nitrogen leaching losses and critical nitrogen application rates in dairy pasture systems using a semi-empirical model. *New Zealand Journal of Agricultural Research* 43: 139–147.

Di HJ, Cameron KC. 2002. Nitrate leaching in temperate agroecosystems: Sources, factors and mitigating strategies. *Nutrient Cycling in Agroecosystems* 64: 237–256.

Eckard RJ, Chapman DF, White RE. 2007. Nitrogen balances in temperate perennial grass and clover dairy pastures in south-eastern Australia. *Australian Journal of Agricultural Research*

58: 1167–1173.

Eckard RJ, Franks DR. 1998. Strategic nitrogen fertiliser use on perennial ryegrass and white clover pasture in north-western Tasmania. *Australian Journal of Experimental Agriculture* 38: 155–160.

Errebhi M, Rosen CJ, Gupta SC, Birong DE. 1998. Potato yield response and nitrate leaching as influenced by nitrogen management. *Agronomy Journal* 90: 10–15.

Fariña SR, Garcia SC, Fulkerson WJ. 2011. A complementary forage system whole-farm study: forage utilisation and milk production. *Animal Production Science* 51: 460–470.

Fessehazion MK, Stirzaker RJ, Annandale JG, Everson CS. 2011. Improving nitrogen and irrigation water use efficiency through adaptive management: A case study using annual ryegrass. *Agriculture, Ecosystems and Environment* 141: 350–358.

Fulkerson B. 2007. Kikuyu grass (*Pennisetum clandestinum). Future Dairy. Tech Note* 1–7.

Fulkerson WJ, Donaghy DJ. 2001. Plant-soluble carbohydrate reserves and senescence - Key criteria for developing an effective grazing management system for ryegrass-based pastures: A review. *Australian Journal of Experimental Agriculture* 41: 261–275.

Fulkerson WJ, Lowe KF. 2011. Forages and Pastures | Grazing Management. In: Fuquay JW, Fox PF, McSweeney PLH (eds), *Encyclopedia of Dairy Science* (2nd edn). Academic Press. pp. 594–601.

Fulkerson WJ, Slack K, Havilah E. 1999. The effect of defoliation interval and height on growth and herbage quality of kikuyu grass (*Pennisetum clandestinum*). *Tropical Grasslands* 33: 138–145.

Garcia SC, Islam MR, Clark CEF, Martin PM. 2014. Kikuyu-based pasture for dairy production: A review. *Crop and Pasture Science* 65: 787–797.

Gilliland TJ, Farrell AD, McGilloway D, Grogan D. 2010. The effect of grass species on nitrogen response in grass clover swards. *Advances in Animal Biosciences* 1: 146.

Good AG, Shrawat AK, Muench DG. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends in Plant Science* 9: 597–605.

Harris SL, Clark DA, Waugh CD, Clarkson FH. 1996. Nitrogen fertiliser effects on white clover in dairy pastures. *Special Publication-Agronomy Society of New Zealand* 11: 119–124.

Haynes RJ, Williams PH. 1993. Nutrient Cycling and soil fertility in grazed pature ecosystem.

Advances in Agronomy 49: 119–199.

Ju X, Lui X, Zhang F, Roelcke M. 2004. Nitrogen Fertilization, Soil Nitrate Accumulation, and Policy Recommendations in Several Agricultural Regions of China. *Ambio* 33: 300–305.

Labuschagne J, Hardy MB, Agenbag GA. 2006. The effects of strategic nitrogen fertiliser application during the cool season on perennial ryegrass-white clover pastures in the Western Cape Province 2. Dry matter production. *South African Journal of Plant and Soil* 23: 253–261.

Ledgard SF, Penno JW, Sprosen MS. 1999. Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. *Journal of Agricultural Science* 132: 215–225.

Ledgard S, Schils R, Eriksen J, Luo J. 2009. Environmental impacts of grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research* 48: 209–226.

López-Garrido R, Madejón E, Murillo JM, Moreno F. 2011. Short and long-term distribution with depth of soil organic carbon and nutrients under traditional and conservation tillage in a Mediterranean environment (southwest Spain) *Soil Use and Management* 27:177–185.

Lowe KF, Fulkerson WJ, Walker RG, Armour JD, Bowdler TM, Slack K, Knight RI, Moody PW, Pepper PM. 2005. Comparative productivity of irrigated short-term ryegrass (*Lolium multiflorum*) pasture receiving nitrogen, grown alone or in a mixture with white (*Trifolium repens*) and Persian (*T. resupinatum*) clovers. *Australian Journal of Experimental Agriculture* 45: 21–39.

Lowe KF, Hume DE, Fulkerson WJ. 2011. Forages and Pastures | Perennial Forage and Pasture Crops – Species and Varieties. In: Fuquay JW, Fox PF, McSweeney PLH (eds), *Encyclopedia of Dairy Science* (2nd edn). Academic Press 576–585.

Marais JP. 2001. Factors affecting the nutritive value of kikuyu grass. *Tropical Grasslands* 35: 65–84.

Marais JP, Figenschou DL, Woodley GAJ. 1990. Energy deficiency in kikuyu grass containing high levels of nitrogen. *South African Journal of Animal Science* 20: 16–20.

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A. 2010. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Annals of Botany* 105: 1141–1157.

Meeske R, Rothauge A, Van Der Merwe GD, Greyling JF. 2006. The effect of concentrate supplementation on the productivity of grazing Jersey cows on a pasture based system.

South African Journal of Animal Sciences 36: 105–110.

Miles N, Thurtell L, Riekert S. 2000. Quality of Kikuyu herbage from pastures in the Eastern Cape coastal belt of South Africa. *South African Journal of Animal Science* 30: 85–86.

Nixon SW. 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia* 41: 199–219.

NRC (National Research Council). 2001. *Nutrient requirements of dairy cattle*. National Academy Press: Washington, DC.

Pembleton KG, Rawnsley RP, Burkitt LL. 2013. Environmental influences on optimum nitrogen fertiliser rates for temperate dairy pastures. *European Journal of Agronomy* 45: 132–141.

Peoples MB, Baldock JA. 2001. Nitrogen dynamics of pastures: nitrogen fixation inputs, the impact of legumes on soil nitrogen fertility, and the contributions of fixed nitrogen to Australian farming systems. *Australian Journal of Experimental Agriculture* 41: 327–346.

Radostits OM, Gay CC, Hinchcliff KW, Constable PD. 2006. Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Saunders Ltd.

Reeves M, Fulkerson WJ, Kellaway RC. 1996. Forage quality of kikuyu (*Pennisetum clandestinum)*: the effect of time of defoliation and nitrogen fertiliser application and in comparison with perennial ryegrass (*Lolium perenne*). *Australian Journal of Agricultural Research* 47: 1349–1359.

Roten RL, Fourie J, Owens JL, Trethewey JAK, Ekanayake DC, Werner A, Irie K, Hagedorn M, Cameron KC. 2017. Urine patch detection using LiDAR technology to improve nitrogen use efficiency in grazed pastures. *Computers and Electronics in Agriculture* 135: 128–133.

Sanderson MA, Rotz CA, Fultz SW, Rayburn EB. 2001. Estimating forage mass with a commercial capacitance meter, rising plate meter, and pasture ruler*. Agronomy Journal* 93: 1281–1286.

Schlueter D, Tracy B. 2011. Sowing Method Effects on Clover Establishment into Permanent Pasture. *Agronomy Journal* 104: 1217–1222.

Silva RG, Cameron KC, Di HJ, Hendry T. 1999. A lysimeter study of the impact of cow urine, dairy shed effluent, and nitrogen fertiliser on nitrate leaching. *Australian Journal of Soil Research* 37: 357–369.

Sinclair K, Beale PJ. 2010. Critical factors influencing no-till establishment of short-term ryegrass (*Lolium multiflorum*) into a kikuyu (*Pennisetum clandestinum*) pasture. *Crop and Pasture Science* 61: 192–200.

Swanepoel PA, du Preez CC, Botha PR, Snyman HA, Habig J. 2014a. Soil quality characteristics of kikuyu-ryegrass pastures in South Africa. *Geoderma* 232–234: 589–599.

Swanepoel PA, Botha PR, Snyman HA, Preez CC. 2014b. Impact of cultivation method on productivity and botanical composition of a kikuyu-ryegrass pasture. *African Journal of Range and Forage Science* 31: 215–220.

Swanepoel PA, Habig J, Preez CC, Botha PR, Snyman HA. 2014c. Biological quality of a podzolic soil after 19 years of irrigated minimum-till kikuyu-ryegrass pasture. *Soil Research* 52: 64–75.

Swanepoel PA, du Preez CC, Botha PR, Snyman HA. 2015. A critical view on the soil fertility status of minimum-till kikuyu–ryegrass pastures in South Africa. *African Journal of Range and Forage Science* 32: 113–124.

Swanepoel PA, Habig J, Du Preez CC, Botha PR, Snyman HA. 2013. Physical quality of a podzolic soil following 19 years of irrigated minimum-till kikuyu-ryegrass pasture. *Soil and Tillage Research* 133: 10–15.

Thompson RB, Fillery IRP. 1998. Fate of urea nitrogen in sheep urine applied to soil at different times of the year in the pasture–wheat rotation in south Western Australia. *Australian Journal of Agricultural Research* 49: 495–510.

Thomson NA, Upsdel MP, Hooper R, Henderson H V, Blackwell MB, Mccallum DA, Hainsworth RJ, Macdonald KA, Wildermoth DD, Bishop-Hurley GJ, Penno JW. 2001. Development and evaluation of a standardised means for estimating herbage mass of dairy pastures using the rising plate meter. In: *Proceedings of the New Zealand Grassland Association* 63: 149–157.

van der Colf J. 2011. The production potential of Kikuyu (*Pennisetum clandestinum)* pastures over-sown with Ryegrass (*Lolium* spp.). MSc(Agric) thesis, University of Pretoria, Pretoria, South Africa.

van der Colf J, Botha PR, Meeske R, Truter WF. 2015. Seasonal dry matter production , botanical composition and forage quality of kikuyu over-sown with annual or perennial ryegrass. *African Journal of Range and Forage Science* 33(2): 133-142.

Varvel GE, Schepers JS, Francis DD. 1997. Ability for In-Season Correction of Nitrogen Deficiency in Corn Using Chlorophyll Meters. *Soil Science Society of America Journal* 61: 1233–1239.

Williams D, Baruch Z. 2000. African grass invasion in the Americas: ecosystem consequences and the role of ecophysiology. *Biological Invasions* 2: 123–140.

Chapter 2 Literature review

2.1 The pedospheric nitrogen cycle

The outer layer of the earth, which includes the soil and processes involving soil, is known as the pedosphere. It is the area where the lithosphere, hydrosphere, atmosphere and biosphere interact (Lal 2012) as is summarised in Figure 2.1.

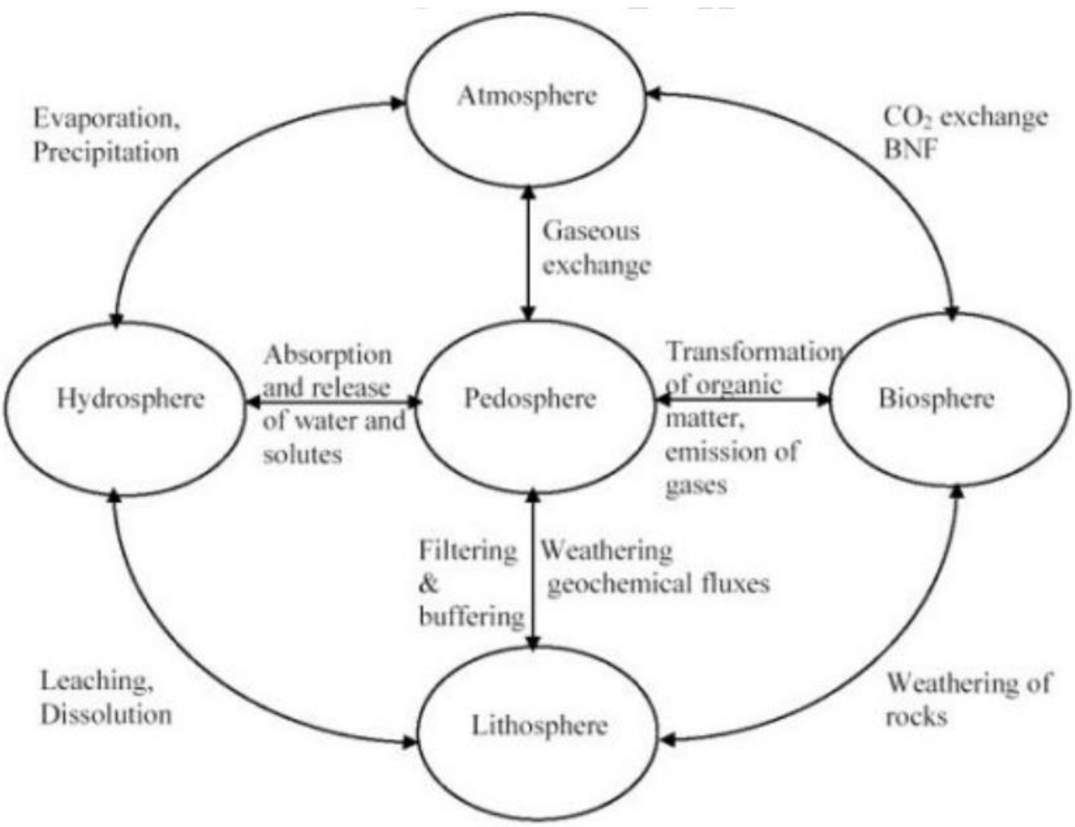

Figure 2.1: Interaction between Soil and the Environment (Lal 2012).

The focus of this review will be on nitrogen (N) and the N cycle. The N cycle takes place in all the above mentioned spheres and is comprised of reactions between both biotic and abiotic components, as illustrated in Figure 2.2 and confirmed by Schipper et al. (1996).

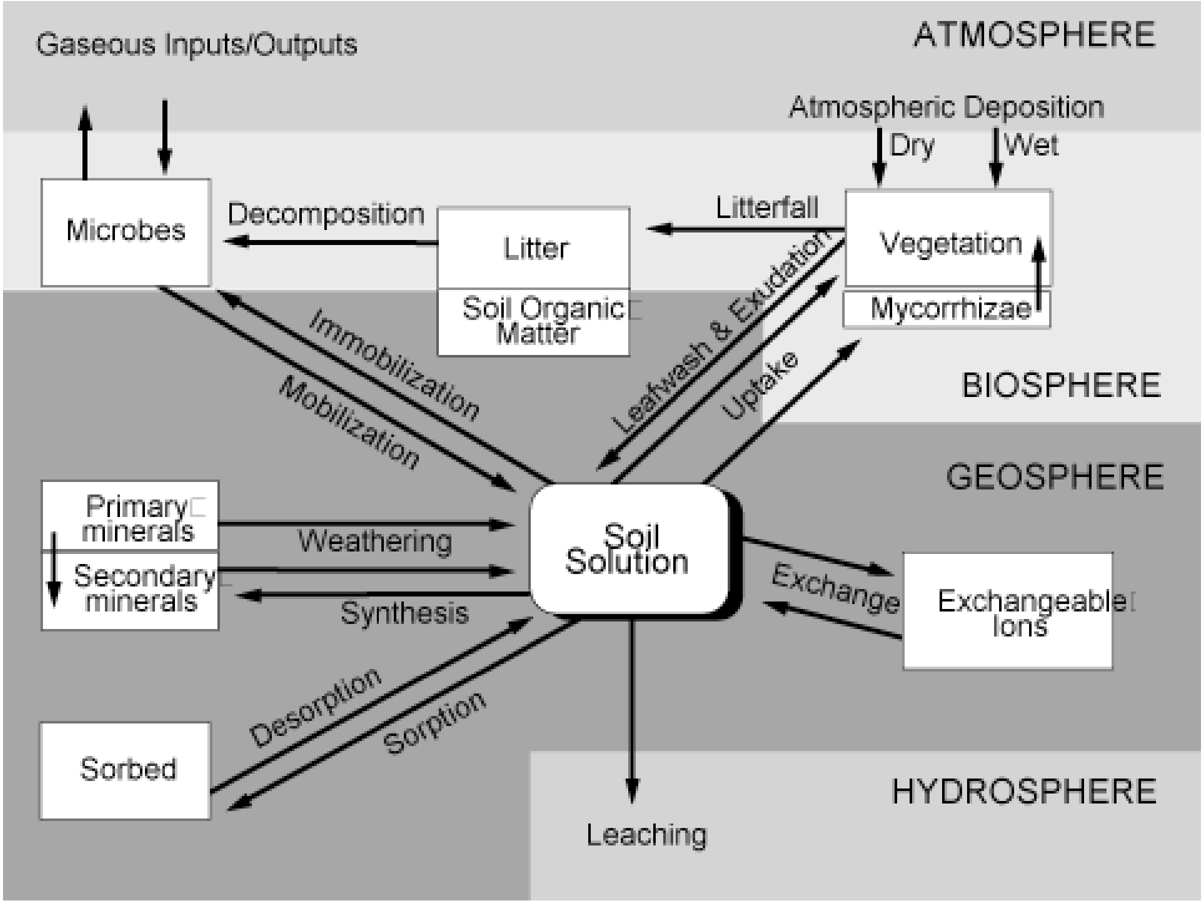

Figure 2.2: Nutrient cycles consist of a series of interrelated processes occurring within and between the atmosphere, hydrosphere, biosphere, and geosphere. Each nutrient is linked through a set of specific interconnected steps that ultimately lead to a series of cyclic pathways (Dahlgren 1998).

From Figure 2.1 and Figure 2.2, the complexity of the N cycle is evident. An overview of the main N reactions regarding agriculture and more specifically pasture-based dairy systems, in the pedosphere and biosphere and the interactions will be the focus of this discussion.

Nitrogen addition to the soil can occur in various ways. Either abiotic, in the form of wet or dry deposition, or by means of biotic N fixation. Lightning is a form of abiotic N fixation (Galloway 1998; Gage 2004; Good et al. 2004; Gruber and Galloway 2008) by which dinitrogen gas is fixed in the presence of oxygen to form N oxides (Hill et al. 1980; Öpik and Rolfe 2005). Tie et al. (2002) reported that from simulations, the suggested global N oxides production by lightning are 7 Tg N yr^{-1}. The N oxides may then react with water to form nitric acid and end up in the soil as nitrates (Postgate 1974) through the process of wet deposition. Precipitation is an example of wet deposition (Ortega et al. 2016). In China, it was estimated that total wet and dry deposition on two agricultural sites varied between 70.7 and 75.5 kg N ha^{-1} yr^{-1} (Pan et al. 2012). Pan et al. (2012) also determined that wet deposition comprised between 29.3 and 35.1% of the total N deposition. Dry deposition refers to dry particles that are covered with N, such as dust and gasses that is deposited onto the soil (Jarvis et al. 1996; Cassman et al. 2002; Almasri and Kaluarachchi 2004; Clark and Kremer 2005; Pan et al. 2012). According to the authors, the estimation of dry deposition is challenging as wind and soil cover, for example, will have an effect on the accuracy of measurements. Dry deposition, on a dairy farm, estimated by simulations was in the range of 5.6 – 14.6 kg N ha^{-1} yr^{-1} (Almasri and Kaluarachchi 2004).

An example of biotic N fixation is the process during which diazotrophs (Piceno and Lovell 2000) fix atmospheric dinitrogen to ammonia (Zahran 1999). An example of a diazotroph organism is the *Rhizobium* genus bacteria that is associated with nodules on the roots of legumes (Sprent and James 2007). The process whereby N is acquired is known as biological N fixation (Jarvis et al. 1996; Di and Cameron 2002; Gage 2004; Rennenberg et al. 2009). In a review article by Ledgard and Steele (1992), it was reported that various authors found biological N fixation rates of between 55 to 342 kg N ha^{-1} yr^{-1} in legume-grass pastures under grazing. Nitrogen additions to the soil can also be from animals in the form of excreta (Hatch et al. 2000; Shepherd and Chambers 2007) and from plants in the form of decomposing material, such as roots, shoots and leaf litter (Haynes and Williams 1993; Hatch et al. 2000; Jones et al. 2005; Rennenberg and Dannenmann 2015). Nitrogen additions from excreta were in the range of 41 to 63 kg N ha^{-1} yr^{-1} when no N fertilisers were applied, and increased to ranges of between 72 to 91 kg N ha^{-1} yr^{-1} under 410 kg N ha^{-1} yr^{-1} fertilisation (Ledgard et al. 1999, Ledgard et al. 2009). The aforementioned additions of N to the soil all contribute to the soil organic N pool. Humans also contribute to the organic N pool through sewage while inorganic fertiliser applications contribute to the inorganic soil N pool (Eckard et al. 2007). The manufacturing of inorganic N fertiliser, or industrial N fixation, where atmospheric dinitrogen is converted to ammonia with hydrogen, is produced by the Haber-Bosch process (Bohlool et al. 1992; Postgate 1998; van Breemen 2002; Gruber and Galloway 2008; Ahmad et al. 2013). It is the inorganic N pool that is plant available and consists mainly of ammonium and nitrate ions (Öpik and Rolfe 2005).

The N cycle is maintained by a flux between reactions in the soil and atmosphere. In the soil, such reactions are amongst others mineralisation, which releases N from organic compounds rendering it available for plant use, and immobilisation, whereby N is bound into organic forms (Brady and Weil 2002).

2.1.1 Mineralisation and immobilisation

Mineralisation and immobilisation is processes in the soil which occur simultaneously and is thus discussed together. Mineralisation is the process during which micro-organisms release N in the form of ammonium ions from organic compounds (Brady and Weil 2002). During this process, the enzymes urease and amidase are important (Das and Varma 2011), since they catalyse the hydrolysis of urea (Roscoe et al. 2000; Cartes et al. 2009) and amides (Frankenberger 1980, Frankenberger and Tabatabai 1982) respectively, to form amongst others ammonium. Immobilisation is the conversion of mineral N, such as nitrate and ammonium, into organic forms (Brady and Weil 2002).

The process whereby ammonium ions are released from organic compounds via mineralisation is known as ammonification. The ammonium ions can undergo nitrification, which refers to the reduced N compound being oxidised to nitrites and then to nitrates through action of nitrifying bacteria (Nitrosomonas and Nitrobacter) (Almasri and Kaluarachchi 2004). Mineralisation is therefore the combined result of ammonification and nitrification. An overview of the importance of both organic and inorganic N to plant nutrition will be presented in section 2.2.1 . Mineralisation and immobilisation processes are dependent on the Carbon (C):N ratio of the soil, and C:N rate of plant residues (Batjes 1996; Cabrera et al. 2005), soil temperature, moisture in the soil, aeration, pH and biological activity (Bolan et al. 2004).

Usually, cultivated soil in the grassland biome had a lower C:N ratio compared with virgin soil due to loss of C (Mills and Fey 2004; Swanepoel et al. 2014a). The author also reported that the higher C:N ratio in virgin soils indicate a low grazing capacity (Swanepoel et al. 2014a). The author reported C:N values the southern Cape of South Africa to be between 11.1 – 12.1 for 0 – 100, 100 – 200 and 200 – 300 mm soil depth under cultivated dairy pastures (Swanepoel et al. 2014a). Pasture in the USA have been reported to have a C:N ratio of 21 (Franzluebbers et al. 2000). For optimal soil quality of pasture a C:N ratio of 8 to 12 is optimal (Sparling et al. 2008). Tainton (2000) also confirmed that well managed pasture soil has a C:N ratio of 10. The soil C:N ratio decrease with increasing fertiliser N (Graham et al. 2002; Parfitt et al. 2012) resulting in mineralisation (Zhang et al. 2012; Hanan et al. 2016).

High C:N ratios of plant residues indicate that the requirements of micro-organisms are met, while low C:N ratios of plant residues indicate that the micro-organisms will require additional N (Hadas et al. 2004; Silva et al. 2005, Robertson and Groffman 2015). Materials with low C:N ratios will therefore result in mineralisation, while materials with high C:N ratios would require additional mineral N to be incorporated into microbial biomass, termed immobilisation (Robertson and Groffman 2015). A higher N content in microbial biomass is correlated with higher mineralisation rates (D'Haene et al. 2008). C_4 grasses had a lower nutritional quality and an increased C:N ratio compared to C_3 grasses (Soussana and Lüscher 2006) resulting in immobilisation. Legumes has a lower C:N ratio than grasses (Andrews et al. 2007) and instigate mineralisation. Dung, however, increase immobilisation rates in fertilised soils because it is a source of available C (Hatch et al. 2000). A C deficiency is generally observed when the dissolved organic matter C:N ratios are between 4.9 – 12.7 (Huygens et al. 2016). Immobilisation of ammonium takes place first until it is exhausted, after which nitrate will be immobilised (Recous and Mary 1990). This is in contrast with results obtained by Burger and Jackson (2003) who found that in some agricultural soils (organic-tomato/maize/legume cover crop and conventional- tomato/wheat), microbial nitrate immobilisation was more than ammonium immobilisation.

Mineralisation by means of ammonification has been found to increase with an increase in temperature. Thangarajan et al. (2015) reported that mineralisation rates were higher at 37°C than at 24°C and 18°C. Furthermore, soil temperatures between 4°C and 10°C have been found to not allow for adequate mineralisation to fulfil the requirement of the pasture plants (Sun et al. 2008; Pembleton et al. 2013). At a temperature of 25°C, the main limitation is the microbial ability to diffuse substrate to metabolically active cells (Zak et al. 1999; Wang et al. 2006), therefore matrix potential is important. Some authors concluded that temperature only had an indirect effect on mineralisation through affecting the microbial activity (Cookson et al. 2006; Qiu et al. 2018). Nitrification rate as affected by temperature has been found to be more complex and was dependent on both the pH of the soil and the various N sources available (Thangarajan et al. 2015). In various studies, nitrification was increased as the pH rose from 4 to 8, but declined below 4 and above 8 (Ste-Marie and Paré 1999; Kyveryga et al. 2004; Thangarajan et al. 2015; Hanan et al. 2016).

According to Zak et al. (1999), mineralisation rates decreased the most as matrix potentials decreased of -0.01 to -0.30 MPa at 25°C, and this decrease in mineralisation rates was smaller as the temperature decreased. A flush in the levels of ammonium and a decrease in nitrate levels is seen when flooding conditions are observed in laboratory experiments (Alaoui-Sossé et al. 2005). In well drained soils, where water filled the pore space of soil up to 60% (Brady and Weil 2002) or water content exceeded field capacity (Scowcroft et al. 2004), nitrification rates are the highest. At 12 h and 120 h after rewetting the soil, microbes assimilated more nitrate compared to ammonium on both conventional and organic managed soils (Burger and Jackson 2003). However, this effect is restricted in soils with a high clay content as it protect compounds from microbial attack (Brady and Weil 2002). On kikuyu pastures, high rates of fertiliser N, and warm and moist conditions led to an increase in mineralisation (Marais et al. 1987).

Tillage has been found to cause a flush of mineralisation (Jarvis et al. 1996; Matlou and Haynes 2006; Fulkerson et al. 2011). This is due to the N that is generally protected in the soil organic N pool becoming available for microbial breakdown (Kristensen et al. 2003) since organisms responsible for nitrification need oxygen to produce nitrite and nitrate (Heldt and Piechulla 2005). However, no or minimum-tillage provides a better environment for microorganisms as was evident in a study by Balota et al. (2004) where no tillage resulted in a significantly higher microbial biomass carbon compared to conventional tillage. Balota et al. (2004) suggested that residues on the surface of a no tillage system could be used as substrate by microbes, while also providing lower soil temperatures and improved water holding capacity among others.

When ammonium and nitrate are not used by the plants it is either immobilised or lost to the environment as volatilisation, denitrification and leaching (Cassman et al. 2002). Management should thus focus on how to optimise mineralisation and how to ensure that mineralised N can be most efficiently utilised within a production system.

2.1.2 Volatilisation

During volatilisation, ammonium is converted to ammonia and gaseous losses occur. Volatilisation often occurs after fertiliser application (McKenzie and Tainton 1993) and from cow urine (Thompson and Fillery 1998) and dung. Ledgard et al. (1999) found that losses through volatilisation from animal excreta were low, around 3-8% on pasture, while losses increased between two and five times as the N fertiliser inputs increased from 0 to 400 kg N ha^{-1} yr^{-1}. Losses through volatilisation due to fertiliser application are also confirmed by other authors (Yerokun 1997; Tian et al. 2000). McKenzie and Tainton (1993) concluded that fertiliser should be applied during cool periods of the day (Black et al. 1985) and when low wind is present to minimise losses through volatilisation (Fessehazion et al. 2012). Volatilisation potentially increases when sward height is 30 mm compared to 100 mm (McKenzie and Tainton 1994), an indirect result of elevated CO_2 (at elevated CO_2, a higher portion of N are allocated to urine N, resulting in higher volatilization losses) (Allard et al. 2003) and with broadcasting compared to incorporating fertilser into the soil (Yerokun 1997). Different studies have different views on wether an increase in soil mositure content decrease (Yerokun 1997) or increase volatilisation rates (McGarry et al. 1987). An increase in pH results in ammonia loss (Black et al. 1985).

2.1.3 Denitrification

Within grazing systems, an undesirable loss of N to the atmosphere often occurs when denitrifying bacteria convert nitrate back into gaseous nitrous oxide and dinitrogen (Ledgard et al. 1999, Almasri and Kaluarachchi 2004). Denitrifying organisms are anaerobic, utilising C as an electron donor and nitrate as the electron acceptor when oxygen is not available (Jarvis et al. 1996; Robertson and Groffman 2015). The optimum temperature for these reactions is 25 to 35°C , but can still take place between 2 and 50°C (Brady and Weil 2002). Denitrification losses were recorded to be between 3 and 7 kg N ha^{-1} yr^{-1} in clover – perennial ryegrass pastures receiving no N (Ruz-Jerez et al. 1994; Ledgard et al. 1999; Luo et al. 2000; Eckard et al. 2007). This denitrification losses increased up to 10 times when the pasture were fertilised with 200 to 400 kg N ha^{-1} yr^{-1} (Ledgard et al. 1999; Eckard et al. 2007). In an article by Luo et al. (1999) that studied the regulation of denitrification in a pasture soil, confirmed that nitrate was the main determinant for denitrification. Denitrification was also influenced was by soil water content that allowed the mobility of the nitrate ions (Luo et al. 1999). Luo et al. (2000) confirmed that soil moisture content was the main limiting factor during dry warm seasons to affect the denitrification rates. Waterlogged soil is prone to denitrification and low rates of mineralisation (Shepherd and Chambers 2007; Fulkerson et al. 2011).

2.1.4 Leaching

Nitrogen in the form of nitrate has a high mobility in the soil since it consists of negatively charged ions and cannot be adsorbed to colloids that are also negatively charged. This in turn, causes nitrate to leach easily into the ground water (Brady and Weil 2002; Almasri and Kaluarachchi 2004). Concentrations of nitrate were high in soils that were managed as productive grasslands (Bardgett et al. 2003). Nitrate pools have been affected by topography, with more nitrates being leached from slopes compared to drainage bottoms (Scowcroft et al. 2004). A major source of nitrate leaching is cow urine (Christensen et al. 2010; Vogeler et al. 2016) and animal waste application from intensive grazing (Almasri and Kaluarachchi 2004). The application of N has an increased effect on leaching. Leaching losses from a clover ryegrass pasture that resulted from 0 kg N were varied between 20 and 74 kg N ha^{-1} yr^{-1}. These losses increased to between 100 and 204 kg N ha^{-1} yr^{-1} when the N application rate increased to 400 kg N ha^{-1} yr^{-1} (Ledgard et al. 1999).

2.2 Nitrogen and growth characteristics of kikuyu and ryegrass species

2.2.1 The role of nitrogen in plant metabolism

Nitrogen is among the primary macronutrients required in relatively large quantities by plants as it is essential for growth and development processes (Öpik and Rolfe 2005). Proteins, such as enzymes, coenzymes and hormones, are chains of combined amino acids that have a C skeleton and amide functional groups originating from ammonia (Brady and Weil 2002, Hofman and Van Cleemput 2004). Nitrogen is also involved in the cell nuclei and carbohydrate utilisation. Ammonium and nitrate are the primary forms in which N are used by plant. Ammonium as a sole N source in plant cells can become toxic to ammonium sensitive species (Britto and Kronzucker 2002). In a review article by Britto and Kronzucker (2002), ammonium sensitive domesticated plant species were listed and included amongst others barley. White clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*) were among the ammonium tolerant species (Belastegui-Macadam et al. 2007). To prevent toxicity, the plant needs to incorporate it into organic compounds through the glutamine synthetase/glutamate synthetase system (Guo et al. 2007). Ammonium may be converted into the amide group of glutamine (Forde and Lea 2007), a plant amino acid that is a product for synthesis of various other amino acids and a principle form of N storage (Masclaux-Daubresse et al. 2010). The soil organic N pool is not as readily available for plants to assimilate into tissues compared to mineral forms (Cassman et al. 2002), however it has been shown that some grass species can use forms of organic N, especially those commonly found on low fertility soil like *Agrostis capillaris* (Weigelt et al. 2003). The two other species that was evaluated was *Holcus lanatus* and *Lolium perenne.* A review by Jones et al. (2005) reported that a large amount of dissolved organic N are available to plants, but that evidence of uptake is still lacking.

Nitrogen fertiliser application must be accompanied by adequate moisture to firstly dissolve the N into useable components such as ammonium and nitrate. The moisture is also needed to facilitate the movement of the nutrients to the root zone for uptake. If the topsoil is dry, the growth of the grass plant may be limited due to restricted nutrient movement (Miles 1997). Effectiveness of production in agricultural systems is dependent on the rate of N turnover, which is determined by the availability soil moisture and competition for inorganic N (Postgate 1974; Høgh-Jensen and Schjoerring 1997; Gruber and Galloway 2008).

2.2.2 Kikuyu

2.2.2.1 Overview and management

Kikuyu, a C_4 subtropical grass (Neal et al. 2007) is a pasture species which is well-adapted to the southern Cape region of South Africa where milk production is a dominant enterprise (Botha 2009). This perennial grass is likely to form a dense mat of fibrous material under mowing or grazing (Mears 1970). Kikuyu has vigorous growing abilities and can be propagated through both seed, below ground rhizomes or above ground stolons (Marais 2001; Botha 2009). Kikuyu has the ability to be deep rooted, which allows it to be drought tolerant (Marais 2001) and also aid in preventing erosion (Radhakrishnan et al. 2006). Kikuyu shows resilience under poor management (Miles et al. 2000), and tends to be unaffected by competition from weeds (Pearson et al. 1985). Kikuyu has the ability to increase soil organic matter as was evident in a study by Swanepoel et al. (2014a; 2015), where soil under long term kikuyu pasture had a higher organic matter status compared to virgin soil. The reasons for this higher organic matter status is minimum-tillage, organic matter cycling by grazing animals and also the abundance of root material and herbage production under such a pasture system (Matlou and Haynes 2006).

Grazing goals of C_4 tropical grasses should aim to optimise quality of the pasture (Fulkerson and Lowe 2011). The grazing of kikuyu should commence at the 4.5 leaves per tiller stage (Reeves et al. 1996) with intervals that may range from 12 days in midsummer to 35 days in late autumn (Fulkerson et al. 1998). During late autumn and winter, defoliation or grazing management may also be determined by over-sown species in the pasture and to prevent competition by kikuyu (Fulkerson et al. 1999).

It is reported that kikuyu requires N fertiliser to significantly increase herbage production with application rates up to 500 kg N ha^{-1} yr^{-1} (Miles 1997). Therefore grazing should also take into account the time of fertiliser application to prevent nitrate poisoning in grazing animals since nitrate concentration is high after fertilising (Bolan and Kemp 2003).

2.2.2.2 Growth requirements

2.2.2.2.1 Temperature requirements

Various temperatures have been outlined as the minimum and maximum growth temperatures for kikuyu, including a range of 24 to 32°C by Murtagh (1988a), while Marais (2001) reported in a review article an optimum growing temperature of between 16 and 21°C. Marais (2001) also reported that growth will be restricted at minimum temperatures of below 8°C . Kikuyu becomes increasingly dormant as the temperature decrease (Pearson et al. 1985). At low night and high day temperature, the growth rate of kikuyu increase resulting in a decrease in quality due to a lowered digestibility (Marais 2001).

2.2.2.2.2 Water requirement

Kikuyu's productivity declines when water is limiting (Murtagh 1988a; Marais 2001; Bell et al. 2011). Rainfall of more than 650 mm is required during the growing season (Cross 1979; Marais 2001). Murtagh (1988b) reported that frequent irrigation will give the best growth response during periods of high evaporation demands (5 mm day^{-1}), while low evaporative demands (2 mm day^{-1}) does not have such a considerable effect on the growth rate. The reason is suggested to be that 50% of the available water in the upper horizon can be removed before growth is affected (Murtagh 1988b). Kikuyu is relatively drought tolerant (Marais 2001). Various authors in the southern Cape have reported using tensiometer readings of between -10 and -25 kPa to schedule irrigation when studying kikuyu-based pasture (Botha et al. 2008a; van der Colf et al. 2015a)

2.2.2.2.3 Soil nutrient requirement

Kikuyu requires fertile soil (Goold 1979; Marais 2001) but can survive under moderately high soil salinity (150 mM NaCl) conditions (Radhakrishnan et al. 2006). A pH($CaCl_2$) of between 4.8 and 6.19 did not affect yield, but kikuyu was negatively affected by pH values lower than 4.36 (Awad et al. 1976).

Many authors have reported on the responsiveness of kikuyu to N fertiliser (Pearson et al. 1985; Miles 1997, Labuschagne and Zulu 2009, Garcia et al. 2014). As a guideline, 300 to 500 kg N ha^{-1} during the growing season should be applied in split dressings (Marais 2001). In Australia, N has been applied commercially at rates up to 300 kg N ha^{-1} yr^{-1} and experimentally up to 1000 kg N ha^{-1} yr^{-1} (Awad et al. 1976). In the southern Cape area in South Africa, N fertiliser applications are in the range of 200 to 600 kg ha^{-1} yr^{-1} (Miles et al. 2000). Also in the southern Cape, rates of between 54 and 60 kg N ha^{-1} grazing cycle^{-1} (10 application) were applied to kikuyu-ryegrass sites (Botha et al. 2008b, Labuschagne and Zulu 2009).

Various methods for the distribution of N applications throughout the year are reported in the literature. For example, Marais (1990) applied a total of 250 kg N ha^{-1} yr^{-1} during the growing season in three equally split dressings in October, December and February. In another study, two applications of 120 kg N ha^{-1} was reported, one during the early growing season (end of October) and one during the end of growing season (end of February) (Marais 1980). In Fulkerson et al. (1998), kikuyu was fertilised with urea at rates of 100 kg ha^{-1} after every grazing during December to May, while Pearson et al. (1985) applied 120 kg N ha^{-1} after every harvest, which was after four weeks in summer and six weeks during winter.

Cross (1979) presented a review of the production of kikuyu in Natal region of SA, comparing the production of previous cutting and grazing trials under different fertilisation regimes. The cutting trials received N applications of between 170 to 240 kg N ha^{-1} yr^{-1} and responded with herbage production that ranged from just below 8 t DM ha^{-1} to above 12 t DM ha^{-1} during the growing season. Cutting trails receiving 200 kg N ha^{-1} yr^{-1} were not significantly different in DM production compared to grazed kikuyu pastures receiving 141 kg N ha^{-1} yr^{-1}. Therefore fertiliser application recommendations based on cutting trials can be reduced by up to 30% when they are utilised for grazed dairy pastures due to recycling and deposition of N through animals. However, Cross (1979) mentioned that reducing the application rate should only occur after two years grazing of N application, so as to allow the soil to build up a level of N, before confidence is placed in this recycled N. Total soil N was recorded on various locations in Highland Sourveld and Natal Mistbelt in South Africa as 0.48, 0.31, 0.49% (Miles 1997). However, Miles (1997) suggested that even with intensive grazing and high rates of N inputs it is difficult to make use of the available N in the soil because it is easily depleted. Even though different genotypes of kikuyu displays different yields, there was no interaction between genotype and N, but rather a trend for different cultivars to display different yields at different locations (Pearson et al. 1985). There is a research need for certainty of N fertilisation for kikuyu pastures since broad ranges (up to 1000 kg N ha^{-1} yr^{-1}) are still reported.

2.2.2.3 Forage quality

On tropical grass pastures, which includes kikuyu, farmers are concerned with adequate N (crude protein (CP)) and phosphorous (P) levels in pasture for the animal diet, but less concerned about dietary potassium (K) deficiency (Cook and Mulder 1984a). As the kikuyu component increase in the pasture from winter to summer, the metabolic energy decreases due to an increase in the DM and neutral detergent fibre (NDF) content (van der Colf et al. 2015b). A range of DM content of kikuyu has been reported, including15% (Kenney et al. 1984), 13.1% (Marais et al. 1990) and 16.8% during autumn (van der Colf et al. 2015b). Swanepoel et al. (2014b) showed that pure kikuyu, where no soil disturbance or over-sowing methods had been applied, had a higher DM content compared to other cultivation methods from April to mid-winter. Dry matter concentration was also reported to be higher in the 0 N treatments compared to when N had been applied (King et al. 2012; Bester 2014). Kikuyu has a higher digestibility compared to what is usually found in other tropical grasses (Jeffery 1971), while the leaf material has a higher nutritional value than the stem material (Marais 1990). Even so, the total non-structural carbohydrate (TNC) content of kikuyu's leaf material is still too low (6.29%) to provide to the animal's nutritional needs which requires up to a 40% TNC of the total DM intake (Marais 1990; Nocek 1997; Miles et al. 2000; NRC 2001). High producing dairy cows utilise the TNC component as an energy source for milk production (NRC 2001). Total non-structural carbohydrates increased from 8.4 to 10.6% as the stubble height increased from 3 to 12 cm (Fulkerson et al. 1999). Other authors have similarly reported TNC values of 5% (Marais et al. 1990) and 9% (Fulkerson et al. 1998). High NDF and overall lower digestibility results in a slow rate of degradation in the rumen which may lead to the low voluntary intake of kikuyu (Reeves et al. 1996) as confirmed in sheep (Joyce 1974). Neutral detergent fibre levels commonly found in kikuyu, was reported by Fulkerson et al. (1999) to be between 43 and 56%, by Miles et al. (2000) to be in the range of 42.3 to 84.0% and by Fulkerson et al. (1998) to be in the range of 40 to 60%. As kikuyu matures, the leaf:stem ratio decreases and thereby decreasing the nutritional value of the sward (Marais 1990). Kikuyu has a low metabolisable energy (Marais et al. 1990) relative to temperate pasture species such as ryegrass. Limitations of kikuyu also include higher cell wall content, low water soluble carbohydrates and nutrient imbalances (Garcia et al. 2014). Excessive N levels in kikuyu pastures cause protein and energy imbalances that reduce the nutritional value of the pasture (Marais et al. 1990).

Garcia et al. (2014) described characteristics of kikuyu resulting in the need of supplementary feeding. Firstly kikuyu, when compared to perennial ryegrass, has high fibre and hemicellulose levels that are reasonably digestible. Secondly, kikuyu contains low levels of TNC, which will affect the amount of dry matter intake as well as the digestibility and therefore also the metabolisable energy (Miles et al. 2000). Kikuyu contains up to 60 g lignin kg^{-1} DM resulting in large amounts of indigestible cell wall carbohydrate (Marais 2001). The third reason for supplementing kikuyu is the unbalanced nature of its mineral content. Miles et al. (2000) reported that in kikuyu the requirement of animals was either matched or exceeded in terms of certain minerals such as K, while copper was undersupplied. Calcium (Ca) is often unavailable to the animal in kikuyu pastures due to the effect of high oxalic acid that binds the Ca (Marais 2001) but kikuyu in the Eastern Cape indicate low levels of oxalate concentrations (Miles et al. 2000). Kikuyu has a high luxury uptake of K, resulting in values exceeding beef and dairy cattle requirements by approximately 10-fold, with whole sward values in the range of 3.04 to 5.60% , stem mean of 5.82% and leaf mean of 4.05% (Marais 1990). It has been reported that kikuyu in the Eastern Cape have a higher Ca content and more favourable Ca:P ratio than other parts of SA and Australia (Miles et al. 2000). Adequate grazing management has also been advocated to manage the Ca:P ratio. When grazing commences after the two leaves per tiller stage, Ca levels rise and CP content fall, but there is also a considerable decrease in organic matter digestibility (Fulkerson et al. 1999). Calcium concentration has been reported to be highest during autumn and winter at 0.34% (Fulkerson et al.

1999). Although the CP content of kikuyu is often reported to be high, N retention was low at 13.7% (2.7 g per sheep) and this could be attributed to low metabolisable energy (Marais et al. 1990). When a decrease in animal production is seen in animals grazing kikuyu, it is most likely due to nitrate levels of the pasture (Marais 1980). Lowered production could be attributable to nitrite that is formed as an intermediate when nitrate is reduced to ammonia by rumen organisms., The nitrite binds with haemoglobin and results in a shortage of oxygen, thus hindering the health of the animal (Marais 1997; 2001).

Supplementary feeding of dairy cattle is expensive (Botha 2009). Knowledge on incorporating supplementation is needed to have a successful operation and prevent financial losses (Botha 2009), as it has been reported that supplementation of high energy concentrate is effective in maintaining milk yield per cow on well managed kikuyu pasture (Meeske et al. 2006; Garcia et al. 2014).

2.2.2.3.1 Protein content

Most of the protein of a kikuyu sward is contained in the leaf material (Marais 1990). Crude protein is calculated by multiplying laboratory determined N content with a factor of 6.25. N application rate affects percentage of N in kikuyu (Pearson et al. 1985). Cook and Mulder (1984b) reported that 0.5 - 1 kg CP ha^{-1} can be expected for every additional kilogram N applied when kikuyu is not actively growing, while an addition of 3 to 4.5 kg CP ha^{-1} may occur in the actively growing period. Furthermore, grass height or pasture yield accumulated can also affect the CP content of kikuyu. With a grass height of 8 to 13 cm, CP value of 10.7% was obtained while grass with height of 20-30 cm had a CP value of 16.9% (Joyce 1974). A decline in CP was also evident as the number of leaves per tiller of kikuyu increased (Reeves et al. 1996; Fulkerson et al. 1999). Crude protein digestibility of grass with a height of 8-13 cm had a value of 54.0%, while 20-30 cm had a value of 66.0% (Joyce 1974). Various authors have reported on the change in CP as a result of the season. Dugmore and du Toit (1988) found a peak in the CP content during autumn in South Africa. In addition, CP values obtained from different studies under different N fertilisation regimes resulted in CP values between 6.2 to 33.3% (Jeffery 1971, Pearson et al. 1985, Marais et al. 1990, Reeves et al. 1996, Fulkerson et al. 1999, Miles et al. 2000, Fourie 2015).
Nitrate may contribute to a further reduction in the digestibility of kikuyu (Marais 1990) and tends to accumulate more in old established kikuyu pastures (Marais et al. 1987). Nitrate also accumulates in the stem material (1.3%) and not in the leaf material, as it did not exceed 0.45% in the latter component (Marais 1990). The form in which fertiliser was applied was found to affect the accumulation of nitrate, with nitrate fertilisation accumulating more nitrate than urea fertilisation (Williams et al. 1991). Optimum stimulation of *in vitro* digestibility occurs at 1 to 1.5 mg g^{-1} nitrate in pasture (Marais, 1980) and therefore, nitrate levels of 5 mg g^{-1} or above are regarded as high and viewed as an anti-quality factor for grazing animals (Marais et al. 1990).

2.2.2.4 Production

In South Africa, kikuyu is important for dairy production in the KwaZulu Natal Midlands, and any improvement in the production of kikuyu will lead to increased profitability (Dugmore and du Toit 1988). Other regions where kikuyu makes a notable contribution to dairy systems include the southern and eastern Cape. Swanepoel et al. (2014b) found annual herbage of 21.29 t DM ha^{-1} with applications of approximately 32 kg N ha^{-1} grazing $cycle^{-1}$ in the southern Cape region. The pure kikuyu pastures produced 6, 1.53, 6.29 and 7.47 t DM ha^{-1} in autumn, winter, spring and summer respectively (Swanepoel et al. 2014b). In the same location Botha et al. (2008b) found that the total annual production of kikuyu was 13.8 t DM ha^{-1} yr^{-1} when N was applied at a rate of 600 kg N ha^{-1} yr^{-1} in 10 split dressings. In another study in Australia, 120 kg N ha^{-1} after each harvest (4 weeks in summer to 6 weeks during winter) produced herbage yields of 23.8, 15.9 and 14.8 t DM ha^{-1} yr^{-1} depending on the location (Pearson et al. 1985). This research found that the highest production occurred at lower latitudes (Pearson et al. 1985).

Lowe et al. (2011) stated that between 12 and 14 t DM ha^{-1} can be expected from well managed kikuyu pastures, while the same was also found by Cook and Mulder (1984a) where 15 t DM ha^{-1} was found under a regime of applying 100 kg N ha^{-1} once every two harvests. In a review article by Garcia et al. (2014), mean annual herbage production of kikuyu based pastures was 12.2 t DM ha^{-1} yr^{-1} while receiving an approximate mean N application of 493.3 kg N ha^{-1} yr^{-1}. In New Zealand, the highest growth rates for kikuyu were found during February and March (Goold 1979) which concur with the findings of Joyce (1974) that production is the highest during summer and autumn months. In Australia, the low production period was found to be during May to October while high producing months were reported to be between November and April (Cook and Mulder 1984b). Similarly, in the southern Cape of South Africa, the highest growth rates were also reported to be during summer and autumn (Botha et al. 2008b).

Kikuyu has a place in dairy systems as part of an optimal mix as reported by Neal et al. (2007), who used a linear programming modelling approach to compute these pasture mixes on a 200 ha farm in Australia. Neal et al. (2007) found that maximum profit could be obtained by having 12 ha under kikuyu over-sown with annual ryegrass and 1 ha under kikuyu over-sown with wheat amongst perennial ryegrass, prairie grass, white clover and plantain. For maximum yield however, all 200 ha should be allocated to kikuyu over-sown with rape. Fertilisation with N has been found to increase the proportion of kikuyu in pastures consisting of multiple species (Pearson et al. 1985).

2.2.2.4.1 Nitrogen use efficiency

In Australia, a yield increase of 3.6 kg DM kg^{-1} N was observed when N application increased from 25 to 50 kg N ha^{-1} in low production period (May to November), while in the high production period (November to April) the increase was 28.2 kg DM kg^{-1} N (Cook and Mulder 1984a). During the low production period, when N application increased from 50 to 100 kg N ha^{-1}, the DM production increased with 5.6 kg DM kg^{-1} N, while in the high production period the DM production increase was 17.8 kg DM kg^{-1} N. Cook and Mulder (1984a) suggested that the most important time for strategic N fertiliser is before and after the summer peak production. In South Africa, the response of kikuyu to N was reported to be in the range of 12.9 to 31.7 kg DM kg^{-1} N (Miles 1997). The author concluded that the large variation was due to N application rate and differences in rainfall in the different years of study. Increasing N application rates and low rainfall resulted in lower agronomic N use efficiencies (ANUE) (Miles 1997). Goold (1979) reported that when kikuyu experienced low temperatures and moisture stress, N often did not have an effect on pasture growth, but it is suggested that the best time for application would be during late winter.

2.2.3 Ryegrass

2.2.3.1 Overview and management

Kikuyu's grazing capacity is increased by over sowing ryegrass into the kikuyu pasture base (Botha et al. 2008a). Swanepoel et al. (2014b) confirmed that the best method for over-sowing ryegrass into kikuyu, to achieve optimum production and quality, was by mulching the kikuyu base and then using a minimum tillage seed drill to plant ryegrass, a method also described by Botha (2003) and van der Colf (2011). The planting date of ryegrass into kikuyu is dependent on the need of the farmer, but if the aim is to overcome fodder flow problems during winter months, it is best to plant Italian ryegrass during February to March in the southern Cape (Botha et al. 2015). The choice of ryegrass species should be based on what is expected of the pasture in terms of production. For example, whether the aim is to increase the pasture quality of kikuyu during summer and autumn, or when high production is required in terms of seasonal production (spring vs. summer) (van der Colf et al. 2015b). The ryegrass should be sown at depths not exceeding 3 cm, with shallower depths being preferable to very deep planting depths. Mulch cover can be a limiting factor for seedling emergence, and residue of 1800 kg DM ha^{-1} resulted in poor emergence and poor persistence (Sinclair and Beale 2010). Grazing of kikuyu prior to planting should thus aim to minimise the accumulation of fibrous residue. No-till establishment combined with shredding of the residue lowered production costs and still resulted in a higher ryegrass production compared to conventional tillage (Bueno et al. 2007). Increasing the seeding rate above 800 pure live seed per square meter did not have an effect on the production (Venuto et al. 2004). At this level, the seeding rate would equate to between 20.2 and 35.8 kg seed ha^{-1} depending on the year and the cultivar choice of Italian ryegrass (Venuto et al. 2004). In a study by Cullen et al. (2008), the grazing management for ryegrass at their various study sites was at two to three leaf stage.

2.2.3.2 Growth requirements

2.2.3.2.1 Temperature requirement

Ryegrass growth is restricted greatly at very low temperatures, together with low solar radiation and waterlogging of soil (Bolland and Guthridge 2007). In Japan, winter air temperatures have been found to restrain the growth of Italian ryegrass (minimum temperature: -1.0°C , maximum temperature: 27.6°C , mean temperature: 10.0°C during October 2006 to April 2007) (Kobayashi et al. 2008). Optimum temperature used in a model was 20°C for annual ryegrass (Cullen et al. 2008). At soil temperatures below 14°C, the uptake of ammonium was found to exceed that of nitrate (Clarkson and Warner 1979) and the result of increased ammonium uptake is lowered root dry weight accumulation (Belastegui-Macadam et al. 2007). Belastegui-Macadam et al. (2007) speculated that the reason might be that ammonium is translocated to the shoot instead of roots. This is an important concept to take into account when oversowing ryegrass into kikuyu, to ensure good root growth.

2.2.3.2.2 Water requirement

Irrigation, by means of overhead sprinklers or movable system with overhead sprays (depending on the site), was scheduled at 50 mm every two weeks in order to prevent water stress (Lowe et al. 2007). In other studies, irrigation was applied as 25 mm per week (Eckard 1989; Harris and Bartholomew 1991). Italian ryegrass in Japan received 576 mm rainfall in the growing period from October to April (Kobayashi et al. 2008). A water deficit in ryegrass was found to result in a lowered N use efficiency (Durand et al. 2010). The water use efficiency increased with an increase in N application (Theron and van Rensburg 1998). Italian ryegrass planted between October and November in Spain (rainfall mainly during October to June period) died off due to heavy rainfall in the growing season, and caused a delay in development (Bueno et al. 2007), indicating that waterlogging may be limiting to ryegrass growth. On the Outeniqua Research Farm (near George, South Africa), it was determined that a ryegrass-clover pasture should be irrigated according to a tensiometer in such a manner that irrigation is applied prior to a reading of -20 kPa (Botha 2002). The author also concluded that on the Escourt soil type in George, it is more effective to apply two 15 mm irrigation compared to one application of 30 mm.

2.2.3.2.3 Soil nutrient requirement

When ryegrass is not grazed, but material removed, special attention should be given to soil K levels. In South Africa, a cutting trial study determined that 518 kg ha^{-1} of K was removed from the soil during April to October (Miles and Hardy 1999). Soil salinity, where the electrical conductivity was 11.2 dS m^{-1} resulted in decreased production of both the roots and shoots (Sagi et al. 1997). The ideal soil pH (KCl) for growth of ryegrass is 5.5 (Miles 1991; Eckard et al. 1995). A prolonged

P deficiency in the soil for longer than six days, negatively affected shoot growth and affected N utilisation as reported by Kim et al. (2003).

Pembleton et al. (2013) reported that the soil mineral N content, in the 0 – 300 mm soil depth of a perennial ryegrass pasture after winter N applications, was different between the N treatments. Soil mineral N content in the soil was 10.5, 21.9 and 54.6 kg N ha^{-1} as a result of total winter N applications of 0, 150 and 300 kg N ha^{-1} respectively (Pembleton et al. 2013). Ryegrass responded well to N fertiliser, since plots receiving no N consistently produced lower yields compared to those fertilised with N (Eckard et al. 1995). Fertilisation regimes differed among authors cited in literature. High inputs were characterized as 420 kg N ha^{-1}, medium as 210 kg N ha^{-1} and low as 105 kg N ha^{-1} by Gilliland et al. (2010). According to Miles (1991), between 200 and 400 kg N ha^{-1} yr^{-1} is required for maximum production. However, according to Eckard (1989) N applications of 300 to 350 kg N ha^{-1} $year^{-1}$ exceeded the N requirements of Italian ryegrass under cutting for the optimum yield of 90%. Harris and Bartholomew (1991) applied 50 kg N ha^{-1} after every harvest. To achieve early spring response, rates of 50 to 60 kg N ha^{-1} during July have been defended (Eckard et al. 1995). Pure Italian ryegrass at different sites were estimated to produce 90% of maximum DM when fertiliser applications was in the range of 50-80 kg N ha^{-1} $month^{-1}$ (Lowe et al. 2005). Seasonal application has also received some attention in research, with certain findings indicating that applying high rates of N during winter (300 kg N ha^{-1}) resulted in losses to the environment (Pembleton et al. 2013).

2.2.3.3 Forage quality

Seasonal DM content for Italian ryegrass sown into kikuyu varied between 12.2 and 15.5% in the first year and between 12.1 and 16.8% during the second year (van der Colf 2011). In pure swards of ryegrass, the DM content varied between years from 18.5% to 15.7% during 2004 and 2005 in spring, while in autumn 2016 the DM content was 13,6 % (Cosgrove et al. 2007). In a study conducted by King et al. (2012), the DM content, during May and June in the years 2009 and 2010, varied between 19.6 and 28.6% under high or low N fertilisation. A lot of pasture species, including Italian ryegrass, takes up K in excess of what the plant needs which results in a decrease in, for example, Ca, magnesium and sodium (Miles 1991). The NDF content of Italian ryegrass varied during the growing season from 35 to 45% (Kobayashi et al. 2008). Similar ranges for NDF were reported by Cosgrove et al. (2007) and King et al. (2012). In the southern Cape of South Africa, where ryegrass was sown into kikuyu, NDF ranges were found to be as low as 46.0 in spring to a high of 70.1% during autumn (Botha et al. 2008b). Van der Colf et al. (2015b) reported NDF values, in the same location, between 37.9% in winter and 57.8% in autumn. As the plant matures the nutritive value decreases (Borreani and Tabacco 1998).

2.2.3.3.1 Protein content

Lowe et al. (2005) found that as N fertilisation increased, N concentration increased in N foliage, while the source of N had little effect on N content. Results have also indicated that as the date of harvest was extended, CP concentration decreased (2.1 g kg^{-1} DM day^{-1}) (King et al. 2012). This was supported by findings from Bryant et al. (2012) that soluble true protein was reduced from the appearance of the second leaf to the appearance of the fourth leaf in perennial ryegrass. In New Zealand the N content was highest in July (winter) and then declined to an ultimate low during in spring (Thom and Prestidge 1996). Various reports from a range of locations and fertiliser applications of CP levels in ryegrass, ranged between 8.3% and 28.3% (Davidson et al. 1997; Bolland and Guthridge 2007; Cosgrove et al. 2007; Kobayashi et al. 2008; Fessehazion et al. 2011; Giambalvo et al. 2011; King et al. 2012).

2.2.3.4 Production

Various factors should be considered when determining the DM production of ryegrasses. Environmental factors, cultivar choices, fertilisation and site differences all have an influence on the herbage production potential and forage quality of ryegrass (Lowe et al. 2007). For example, ryegrass type can greatly influence the production of a sward, with Westerwolds ryegrass found to die out sooner than Italian ryegrass (Harris and Bartholomew 1991). Fertiliser N increased the DM production of ryegrass but the response could have been influenced by the high residual N in soil (King et al. 2012). During early spring (August), treatments that received N applications at six week intervals consistently produced higher compared to four week N application intervals, while at the end of Spring (October – November), the four week applications out produced all other times and rates (Eckard et al. 1995). To ensure a high early spring production, it is validated to apply high rates of N (50 – 60 kg DM ha^{-1}) in July (Eckard et al. 1995). Annual ryegrass produced 14 t DM ha^{-1} receiving 400 kg N ha^{-1} (Davidson et al. 1997). Optimum N application was determined by the N content of the pasture during October to obtain 90% of yield in November by Vogeler and Cichota (2016). The N content were put in categories of 2.0 to 2.4, 2.5 to 2.8, 2.9 to 3.2, 3.3 to 3.6, and 3.7 to 4.0% N and it was determined that it should receive 158, 144, 112, 85 and 59 kg N ha^{-1} respectively (Vogeler and Cichota 2016). Bolland and Guthridge (2007) reported that as more N is applied to mixed ryegrass-clover pastures, ryegrass content increased while clover content decreased. However site can play an important role in the production potential as DM production of 13.5, 11.8 and 15.4 t ha^{-1} yr^{-1} were obtained from different sites (Eckard 1989). In South Africa, Italian ryegrass sown into kikuyu yielded 19.5, 18.4, 17.6 and 16.6 t DM ha^{-1} at different sites and ploidy levels (Harris and Bartholomew 1991). Swanepoel et al. (2014b) reported herbage productions of 20.3 t DM ha^{-1} yr^{-1}. Mean annual grazing capacity on the Outeniqua Research Farm was 6.4 and 5.3 cows ha^{-1} (van der Colf et al. 2015a).

It is evident from previous research that there is a paucity in dairy research which brings together three aspects of pasture dairy production namely fertilisation, seasonal DM production and animal production. It should be important to know what cultivar to use in the specific region, when to fertilise and what the seasonal DM production would be in order to obtain a linear fodder flow for the dairy cows.

2.2.3.4.1 Agronomic N use efficiency

The agronomic N use efficiency (ANUE) of ryegrass was found to be unaffected by treatment and varied greatly between years and location of experiments by Lowe et al. (2005). In addition, a study by Bolland and Guthridge (2007) found that the lowest efficiency was during winter and autumn while the highest efficiency was found in spring. There was generally an inverse relationship between ANUE and N application rate in a study conducted by Lowe et al. (2005), but the differences between treatments were not significant. In a another study, different rates of N did not affect the response to N applied, but the production did vary largely across years and experimental sites (Bolland and Guthridge 2007). Eckard (1989) stated that the low N response often reported in ryegrass might be attributable to high residual N in soils. The response of pasture was 33.9, 23.8, 27.6 and 25.0 kg DM increase per kg N applied and were obtained respectively in response to application rates of 0 to 200 kg N ha^{-1} yr^{-1}. King et al. (2012) reported an average of 9.9 kg DM increase per kg N applied ha^{-1}. Lowe et al. (2005) reported that when N was applied after every cut, instead of every second cut, ANUE was higher.

2.3 References

Ahmad P, Wani MR, Azooz MM, Phan Tran LS (eds). 2013. *Improvement of Crops in the Era of Climatic Changes.* Volume 2, Springer.

Alaoui-Sossé B, Bastien G, Binet P, Toussaint ML, Badot PM. 2005. Influence of flooding on growth, nitrogen availability in soil, and nitrate reduction of young oak seedlings (*Quercus robur* L.). *Annals of Forest Science* 62: 593–600.

Allard V, Newton PCD, Lieffering M, Clark H, Matthew C, Soussana JF, Gray YS. 2003. Nitrogen cycling in grazed pastures at elevated CO2: N returns by ruminants. *Global Change Biology* 9: 1731–1742.

Almasri MN, Kaluarachchi JJ. 2004. Implications of on-ground nitrogen loading and soil transformations on ground water quality management. *Journal of the American Water Resources Association* 40: 165–186.

Andrews M, Scholefield D, Abberton MT, Mckenzie BA, Hodge S, Raven JA. 2007. Use of white clover as an alternative to nitrogen fertiliser for dairy pastures in nitrate vulnerable zones in the UK : productivity, environmental impact and economic considerations. *Annals of Applied Biology* 151: 11–23.

Awad AS, Edwards DG, Milham PJ. 1976. Effect of pH and phosphate on soluble soil aluminium and on growth and composition of kikuyu grass. *Plant and Soil* 45: 531–542.

Balota EL, Filho AC, Andrade DS, Dick RP. 2004. Long-term tillage and crop rotation effects on microbial biomass and C and N mineralization in a Brazilian Oxisol. *Soil and Tillage Research* 77: 137–145.

Bardgett RD, Streeter TC, Bol R. 2003. Soil Microbes Compete Effectively With Plants For Organic-Nitrogen Inputs To Temperate Grasslands. *Ecological Society of America* 84: 1277–1287.

Batjes NH. 1996. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* 47: 151–163.

Belastegui-Macadam XM, Estavillo JM, García-Mina JM, González A, Bastias E, Gónzalez-Murua C. 2007. Clover and ryegrass are tolerant species to ammonium nutrition. *Journal of Plant Physiology* 164:1583–1594.

Bell MJ, Cullen BR, Eckard RJ. 2011. The production of perennial ryegrass and kikuyu pastures in south-eastern Australia under warmer and drier future climate scenarios. In: *International Congress on Modelling and Simulation, 12 – 16 December, Perth, Australia.* pp 753–759

Bester C. 2014. Nitrogen management strategies for mixed pastures grown under irrigation in the Winelands sub-region of the Western Cape. MSc(Agric) thesis, University of Stellenbosch, Stellenbosch, South Africa.

Black AS, Sherlock RR, Smith NP, Cameron KC, Goh KM. 1985. Effects of form of nitrogen, season, and urea application rate on ammonia volatilisation from pastures. *New Zealand Journal of Agricultural Research* 28: 469–474.

Bohlool BB, Ladha JK, Garrity DP, George T. 1992. Biological nitrogen fixation for sustainable agriculture: A perspective. *Plant and Soil Sciences* 141: 1–11.

Bolan NS, Horne DJ, Currie LD. 2004. Growth and chemical composition of legume-based pasture irrigated with dairy farm effluent. *New Zealand Journal of Agricultural Research* 47: 85–93.

Bolan NS, Kemp PD. 2003. A review of factors affecting and prevention of pasture-induced nitrate toxicity in grazing animals. In: *Proceedings of New Zealand Grassland Association* 65: 171–178.

Bolland MDA, Guthridge IF. 2007. Responses of intensively grazed dairy pastures to applications of fertiliser nitrogen in south-western Australia. *Australian Journal of Experimental Agriculture* 47: 927–941.

Borreani G, Tabacco E. 1998. Effects of crop properties, weather conditions and mechanical treatments on the wilting rate of diploid and tetraploid Italian ryegrass for silage. *Grass and Forage Science* 53: 179–188.

Botha PR. 2002. Die gebruik van vogspanningsmeters vir besproeiingskedulering by weidings. In: Weidingskursus Inligtingsbundel 2002. pp 141-146.

Botha PR. 2003. Die produksiepotensiaal van oorgesaaide kikoejoeweiding in die gematigde kusgebied van die Suid-Kaap. PhD thesis, University of the Freestate, Bloemfontein, South Africa.

Botha PR. 2009. Factors influencing the persistence and production potential of kikuyu (*Pennisetum clandestinum*) over-sown with different ryegrass and clover species in the southern Cape. *Elsenburg Joernaal* 3: 4–9.

Botha PR, Meeske R, Snyman HA. 2008a. Kikuyu over-sown with ryegrass and clover: grazing capacity, milk production and milk composition. *African Journal of Range and Forage Science* 25(3): 103–110.

Botha PR, Meeske R, Snyman HA. 2008b. Kikuyu over-sown with ryegrass and clover: dry matter production, botanical composition and nutritional value. *African Journal of Range and*

Forage Science 25(3):93–101.

Botha PR, Zulu LB, van der Colf J, Swanepoel PA. 2015. Production potential of Italian and Westerwolds ryegrass established at different planting dates. *African Journal of Range and Forage Science* 32: 153–159.

Brady NC, Weil RR. 2002. *The Nature and Properties of Soils* (13th edn). Prentice Hall

Britto DT, Kronzucker HJ. 2002. NH_4^+ toxicity in higher plants: a critical review. *Journal of Plant Physiology* 159: 567–584.

Bryant RH, Gregorini P, Edwards GR. 2012. Effects of N fertilisation, leaf appearance and time of day on N fractionation and chemical composition of *Lolium perenne* cultivars in spring. *Animal Feed Science and Technology* 173: 210–219.

Bueno J, Amiama C, Hernanz JL. 2007. No-tillage drilling of Italian ryegrass (*Lolium multiflorum* L.): Crop residue effects, yields and economic benefits. *Soil and Tillage Research* 95: 61–68.

Burger M, Jackson LE. 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. *Soil Biology and Biochemistry* 35: 29–36.

Cabrera ML, Kissel DE, Vigil MF. 2005. Nitrogen Mineralization from Organic Residues: Research Opportunities. *Journal of environmental management* 34: 75–79.

Cartes P, Jara AA, Demanet R, de la Luz Mora M. 2009. Urease activity and nitrogen mineralization kinetics as affected by temperature and urea Input rate in Southern Chilean Andisols. *Journal of Soil Science and Plant Nutrition* 9: 69–82.

Cassman KG, Dobermann AR, Walters DT. 2002. Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management. *Ambio* 31: 132–140.

Christensen CL, Hanly JA, Hedley MJ, Horne DJ. 2010. Reducing nitrate leaching losses by using duration-controlled grazing of dairy cows. *19th World Congress of Soil Science, Soil Solutions for a Changing World* 153–156.

Clark H, Kremer JN. 2005. Estimating direct and episodic atmospheric nitrogen deposition to a coastal waterbody. *Marine Environmental Research* 59: 349–366.

Clarkson DT, Warner AJ. 1979. Relationships between Root Temperature and the Transport of Ammonium and Nitrate Ions by Italian and Perennial Ryegrass (*Lolium multiflorum* and *Lolium perenne*). *Plant physiology* 64: 557–561.

Cook BG, Mulder JC. 1984a. Responses of nine tropical grasses to nitrogen fertilizer under rain-grown conditions in south-eastern Queensland 1. Seasonal dry matter productivity. *Australian Journal of Experimental Agriculture and Animal Husbandry* 24: 410–414.

Cook BG, Mulder JC. 1984b. Responses of nine tropical grasses to nitrogen fertilizer under rain-grown conditions in south-eastern Queensland 2. Concentrations and uptakes of nitrogen, phosphorus and potassium in plant tops. *Australian Journal of Experimental Agriculture and Animal Husbandry* 24: 415–420.

Cookson WR, Marschner P, Clark IM, Milton N, Smirk MN, Murphy D V., Osman M, Stockdale EA, Hirsch PR. 2006. The influence of season, agricultural management, and soil properties on gross nitrogen transformations and bacterial community structure. *Australian Journal of Soil Research* 44: 453–465.

Cosgrove GP, Burke JL, Death AF, Hickey MJ, Pacheco D, Lane GA. 2007. Improved water-soluble carbohydrate in forages: evaluating the potential for grazing dairy cows in NZ. In: *Proceedings of the New Zealand Grassland Association* 69: 179–185.

Cross GW. 1979. The production potential of *Pennisetum clandestinum* in the midlands and on the south coast of Natal. *Proceedings of the Annual Congresses of the Grassland Society of Southern Africa* 14: 53–59.

Cullen BR, Eckard RJ, Callow MN, Johnson IR, Chapman DF, Rawnsley RP, Garcia SC, White T, Snow VO. 2008. Simulating pasture growth rates in Australian and New Zealand grazing systems. *Australian Journal of Agricultural Research* 59: 761–768.

D'Haene K, Vandenbruwane J, De Neve S, Donald G, Salomez J, Hofman G. 2008. The effect of reduced tillage on nitrogen dynamics in silt loam soils. *European Journal of Agronomy* 28: 449–460.

Dahlgren RA. 1998. Effects of forest harvest on stream-water quality and nitrogen cycling in the Caspar Creek watershed. In: *Ziemer, Robert R., technical coordinator. Proceedings of the conference on coastal watersheds: the Caspar Creek story, 6 May 1998; Ukiah, California. General Tech. Rep. PSW GTR-168. Albany, California: Pacific Southwest Research Station, Forest Service, US Department of Agriculture:* Vol. 168: 45-53.

Das SK, Varma A. 2011. Role of enzymes in maintaining soil health. In: Shukla G, Varma A. (eds.). *Soil Enzymology*. Springer-Verlag, Berlin, Germany 25-42.

Davidson TM, Frampton PJ, Orr WN, Silver BA, Martin P, McLachlan B. 1997. An evaluation of kikuyu-clover pastures as a dairy production system. 1. Pasture and diet. *Tropical Grasslands* 31: 1–14.

Di HJ, Cameron KC. 2002. Nitrate leaching in temperate agroecosystems: Sources, factors and mitigating strategies. *Nutrient Cycling in Agroecosystems* 64: 237–256.

Dugmore TJ, du Toit JH. 1988. The chemical composition and nutritive value of kikuyu pasture. *South African Journal of Animal Science* 18: 72–75.

Durand J, Gonzalez-Dugo V, Gastal F. 2010. How much do water deficits alter the nitrogen nutrition status of forage crops? *Nutrient Cycling in Agroecosystems* 88, 231–243.

Eckard RJ. 1989. The response of Italian ryegrass to applied nitrogen in the Natal Midlands. *Journal of the grassland society of Southern Africa* 6: 19–22.

Eckard RJ, Bartholomew PE, Tainton NM. 1995. The yield response of annual ryegrass *(Lolium multiflorum*) to varying nitrogen fertilizer application strategies. *South African Journal of Plant Soil* 12: 112–116.

Eckard RJ, Chapman DF, White RE. 2007. Nitrogen balances in temperate perennial grass and clover dairy pastures in south-eastern Australia. *Australian Journal of Agricultural Research* 58: 1167–1173.

Fessehazion MK, Abraha AB, Everson CS, Truter WF, Annandale JG, Moodley M. 2012. *Water Use and Nitrogen Application for Irrigation Management of Annual Ryegrass and Kikuyu Pasture Production.* WRC Technical Report TT 520/12. Pretoria, South Africa: Water Research Commission (WRC).

Fessehazion MK, Stirzaker RJ, Annandale JG, Everson CS. 2011. Improving nitrogen and irrigation water use efficiency through adaptive management: A case study using annual ryegrass. *Agriculture, Ecosystems and Environment* 141: 350–358.

Forde BG, Lea PJ. 2007. Glutamate in plants: Metabolism, regulation, and signalling. *Journal of Experimental Botany* 58: 2339–2358.

Fourie I. 2015. Production potential of lucerne (*Medicago sativa*) over-sown into kikuyu (*Pennisetum clandestinum*) based pasture. MSc thesis, Nelson Mandela Metropolitan University, George, South Africa.

Frankenberger WT. 1980. Amidase activity in soils. PhD thesis, Iowa State University, Ames, Iowa.

Frankenberger WT, Tabatabai MA. 1982. Amidase and urease activities in plants. *Plant and Soil* 64(2): 153-166.

Franzluebbers AJ, Stuedemann JA, Schomberg HH, Wilkinson SR. 2000. Soil organic C and N pools under long-term pasture management in the Southern Piedmont USA. *Soil Biology and Biochemistry* 32: 469–478.

Fulkerson WJ, Lowe KF. 2011. Forages and Pastures | Grazing Management. In: Fuquay JW, Fox PF, McSweeney PLH (eds), *Encyclopedia of Dairy Science* (2nd edn). Academic Press. pp. 594–601.

Fulkerson WJ, Lowe KF, Hume DE. 2011. Forages and Pastures | Perennial Forage and Pasture Crops - Establishment and Maintenance. In: Fuquay JW, Fox PF, McSweeney PLH (eds), *Encyclopedia of Dairy Science* (2nd edn). Academic Press. pp. 586–593.

Fulkerson WJ, Slack K, Havilah E. 1999. The effect of defoliation interval and height on growth and herbage quality of kikuyu grass (*Pennisetum clandestinum*). *Tropical Grasslands* 33: 138–145.

Fulkerson WJ, Slack K, Hennessy DW, Hough GM. 1998. Nutrients in ryegrass, white cloveer and kikuyu pastures in relation to season and stage of regrowth in a subtropical environment. *Australian Journal of Experimental Agriculture* 38: 277–240.

Gage DJ. 2004. Infection and Invasion of Roots by Symbiotic, Nitrogen-Fixing Rhizobia during Nodulation of Temperate Legumes. *Microbiology and Molecular Biology Reviews* 68: 280–300.

Galloway JN. 1998. The global nitrogen cycle: Changes and consequences. *Environmental Pollution* 102: 15–24.

Garcia SC, Islam MR, Clark CEF, Martin PM. 2014. Kikuyu-based pasture for dairy production: a review. *Crop and Pasture Science* 65: 787–797.

Giambalvo D, Ruisi P, Di Miceli G, Frenda AS, Amato G. 2011. Forage production, N uptake, N_2 fixation, and N recovery of berseem clover grown in pure stand and in mixture with annual ryegrass under different managements. *Plant and Soil* 342: 379–391.

Gilliland TJ, Farrell AD, McGilloway D, Grogan D. 2010. The effect of grass species on nitrogen response in grass clover swards. *Advances in Animal Biosciences* 1: 146.

Good AG, Shrawat AK, Muench DG. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends in Plant Science* 9: 597–605.

Goold GJ. 1979. Effect of nitrogen and cutting interval on production of grass species swards in Northland, New Zealand. *New Zealand Journal of Experimental Agriculture* 7: 353–359.

Graham MH, Haynes RJ, Meyer JH. 2002. Soil organic matter content and quality : effects of fertilizer applications, burning and trash retention on a long-term sugarcane experiment in South Africa. *Soil Biology and Biochemistry* 34: 93–102.

Gruber N, Galloway JNJ. 2008. An Earth-system perspective of the global nitrogen cycle. *Nature* 451: 293–296.

Guo SW, Zhou Y, Gao YX, Li Y, Shen QR. 2007. New Insights into the Nitrogen Form Effect on Photosynthesis and Photorespiration. *Pedosphere* 17: 601–610.

Hadas A, Kautsky L, Goek M, Kara EE. 2004. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. *Soil Biology and Biochemistry* 36: 255–266.

Hanan EJ, Schimel JP, Dowdy K, D'Antonio CM. 2016. Effects of substrate supply, pH, and char on net nitrogen mineralization and nitrification along a wildfire-structured age gradient in chaparral. *Soil Biology and Biochemistry* 95: 87–99.

Harris DI, Bartholomew PE. 1991. The production of four ryegrass cultivars oversown at various seeding rates into irrigated kikuyu. *Journal of the Grassland Society of Southern Africa* 8: 82–85.

Hatch DJ, Lovell RD, Antil RS, Jarvis SC, Owen PM. 2000. Nitrogen mineralization and microbial activity in permanent pastures amended with nitrogen fertilizer or dung. *Biology and Fertility of Soils* 30: 288–293.

Haynes R, Williams P. 1993. Nutrient Cycling and soil fertility in grazed pature ecosystem. *Advances in Agronomy* 49: 119–199.

Heldt H-W, Piechulla B. 2005. Nitrate assimilation is essential for the synthesis of organic matter. *Plant Biochemistry* 273–305

Hill RD, Rinker RG, Wilson HD. 1980. Atmospheric Nitrogen-Fixation by Lightning. *Journal of Atmospheric Sciences* 37: 179–192.

Hofman G, Van Cleemput O. 2004. Soil and plant nitrogen. Paris, France: International Fertilizer Industry Association (IFA).

Høgh-Jensen H, Schjoerring JK. 1997. Interactions between white clover and ryegrass under contrasting nitrogen availability: N_2 fixation, N fertilizer recovery, N transfer and water use efficiency. *Plant and Soil* 197: 187–199.

Huygens D, Díaz S, Urcelay C, Boeckx P. 2016. Microbial recycling of dissolved organic matter confines plant nitrogen uptake to inorganic forms in a semi-arid ecosystem. *Soil Biology and Biochemistry* 101: 142–151.

Jarvis SC, Stockdale EA, Shepherd MA, Powlson DS. 1996. Nitrogen Mineralization in Temperate Agricultural Soils: Processes and Measurements. *Advances in Agronomy* 57: 187–235.

Jeffery H. 1971. Nutritive value of *Pennisetum clandestinum* based pastures in a sub-troplcal environment. *Australian Journal of Experimental Agriculture and Animal Husbandry* 11: 173–177.

Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A. 2005. Dissolved organic nitrogen uptake by plants – An important N uptake pathway? *Soil Biology and Biochemistry* 37: 413–423.

Joyce JP. 1974. Nutritive value of Kikuyu grass. *New Zealand Journal of Agricultural Research* 17, 197–202.

Kenney PA, Black JL, Colebrook WF. 1984. Factors Affecting Diet Selection by Sheep. III Dry Matter Content and Particle Length of Forage. *Australian Journal of Agricultural Research* 35: 831–838.

Kim TH, Jung WJ, Lee BR, Yoneyama T, Kim HY, Kim KY. 2003. P effects on N uptake and remobilization during regrowth of Italian ryegrass (*Lolium multiflorum*). *Environmental and Experimental Botany* 50: 233–242.

King C, McEniry J, Richardson M, O'Kiely P. 2012. Yield and chemical composition of five common grassland species in response to nitrogen fertiliser application and phenological growth stage. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science* 62: 644–658.

Kobayashi H, Takahashi Y, Matsumoto K, Nishiguchi Y. 2008. Changes in nutritive value of Italian Ryegrass (*Lolium multiflorum* Lam.) during overwintering period. *Plant Production Science* 11: 228–231.

Kristensen HL, Debosz K, McCarty GW. 2003. Short-term effects of tillage on mineralization of nitrogen and carbon in soil. *Soil Biology and Biochemistry* 35: 979–986.

Kyveryga PM, Blackmer AM, Ellsworth JW, Isla R. 2004. Soil pH Effects on Nitrification of Fall-Applied Anhydrous Ammonia. *Soil Science Society of America Journal* 68: 545.

Labuschagne J, Zulu B. 2009. Ontwikkeling van stikstofbemestingsnorme vir kikoejoe oorgesaai met raaigras. In: *Proceedings of Outeniqua Research Farm Information Day*. Western Cape Department of Agriculture, South Africa.

Lal R. 2012. Soil and its life support systems. *Agricultural sciences* 1: 1–12.

Ledgard S, Schils R, Eriksen J, Luo, J. 2009. Environmental impacts of grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research* 48: 209–226.

Ledgard SF, Penno JW, Sprosen MS. 1999. Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. *Journal of Agricultural Science* 132: 215–225.

Ledgard SF, Steele KW. 1992. Biological nitrogen fixation in mixed legume/grass pasture. *Plant and Soil* 141: 137-153.

Lowe KF, Bowdler TM, Casey ND, Lowe SA, White JA, Pepper PM. 2007. Evaluating temperate species for the subtropics. 1. Annual ryegrasses. *Tropical Grasslands* 41: 9–25.

Lowe KF, Fulkerson WJ, Walker RG, Armour JD, Bowdler TM, Slack K, Knight RI, Moody PW, Pepper PM. 2005. Comparative productivity of irrigated short-term ryegrass (*Lolium multiflorum*) pasture receiving nitrogen, grown alone or in a mixture with white (*Trifolium repens*) and Persian (*T. resupinatum*) clovers. *Australian Journal of Experimental Agriculture* 45: 21–39.

Lowe KF, Hume DE, Fulkerson WJ. 2011. Forages and Pastures | Perennial Forage and Pasture Crops – Species and Varieties. In: Fuquay JW, Fox PF, McSweeney PLH (eds), *Encyclopedia of Dairy Science* (2nd edn). Academic Press 576–585.

Luo J, Tillman RW, Ball PR. 1999. Factors regulating denitrification in a soil under pasture. *Soil Biology and Biochemistry* 31:913–927.

Luo J, Tillman RW, Ball PR. 2000. Nitrogen loss through denitrification in a soil under pasture in New Zealand. *Soil Biology and Biochemistry* 32:497–509.

Marais JP. 1980. Effect of nitrate and non-protein organic nitrogen levels in Kikuyu (*Pennisetum clandestinum*) pastures on digestibility in vitro. *Agroanimalia* 12: 7–11.

Marais JP. 1990. Relationship between nitrogen and other chemical components in kikuyu grass from long-established pastures. *South African Journal of Animal Science* 20: 147–151.

Marais JP. 1997. 14 Nitrate and Oxalates. *Handbook of Plant Fungal Toxicants*. 205–218

Marais JP. 2001. Factors affecting the nutritive value of kikuyu grass. *Tropical Grasslands* 35: 65–84.

Marais JP, Figenschou DL, Dennison C. 1987. The accumulation of nitrate in Kikuyu grass (*Pennisetum clandestinum* Hochst). South African *Journal of Plant and Soil* 4: 82–88.

Marais JP, Figenschou DL, Woodley GAJ. 1990. Energy deficiency in kikuyu grass containing high levels of nitrogen. *South African Journal of Animal Science* 20: 16–20.

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A. 2010. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Annals of Botany* 105: 1141–1157.

Matlou MC, Haynes RJ. 2006. Soluble organic matter and microbial biomass C and N in soils under pasture and arable management and the leaching of organic C, N and nitrate in a lysimeter study. *Applied Soil Ecology* 34: 160–167.

McGarry SJ, O'Toole P, Morgan MA. 1987. Effects of Soil Temperature and Moisture Content on Ammonia Volatilization from Urea-Treated Pasture and Tillage Soils. *Irish Journal of Agricultural Research* 26: 173–182.

McKenzie FR, Tainton NM. 1993. Pattern of volatilized nitrogen loss from dryland kikuyu pastures after fertilization. *African Journal of Range and Forage Science* 10: 86–91.

McKenzie FR, Tainton NM. 1994. The effect of sward height on volatilized nitrogen from a dryland *Pennisetum clandestinum* Hoscht. pasture. *African Journal of Range and Forage Science* 11: 59–60.

Mears P. 1970. Kikuyu - (*Pennisetum clandestinum*) as a pastures grass - a review. *Tropical Grasslands* 4: 139–152.

Meeske R, Rothauge A, Van Der Merwe GD, Greyling JF. 2006. The effect of concentrate supplementation on the productivity of grazing Jersey cows on a pasture based system. *South African Journal of Animal Sciences* 36: 105–110.

Miles N. 1991. Nutrition of intensive pastures in the summer rainfall areas of southern Africa. *Journal of the Grassland Society of Southern Africa* 8: 39–48.

Miles N. 1997. Responses of productive and unproductive kikuyu pastures to top-dressed nitrogen and phosphorus fertiliser. *African Journal of Range and Forage Science* 14: 1–6.

Miles N, Hardy MB. 1999. Soil fertility management in pasture small-plot trials: potential pitfalls. *African Journal of Range and Forage Science* 16: 101–107.

Miles N, Thurtell L, Riekert S. 2000. Quality of Kikuyu herbage from pastures in the Eastern Cape coastal belt of South Africa. *South African Journal of Animal Science* 30: 85–86.

Mills AJ, Fey M V. 2004. Declining soil quality in South Africa : effects of land use on soil organic matter and surface crusting. *South African Journal of Plant and Soil* 21: 388–398.

Murtagh GJ. 1988a. Factors Affecting the Growth of Kikuyu. I Potential Growth and Nitrogen Supply. *Australian Journal of Agricultural Research* 38: 31–42.

Murtagh GJ. 1988b. Factors affecting the growth of Kikuyu. II Water supply. *Australian Journal of Agricultural Research* 39: 43–51.

Neal M, Neal J, Fulkerson WJ. 2007. Optimal choice of dairy forages in eastern Australia. *Journal of Dairy Science* 90: 3044–59.

Nocek JE. 1997. Bovine Acidosis: Implications on Laminitis. *Journal of Dairy Science* 80(5):1005–1028.

NRC (National Research Council). 2001. *Nutrient requirements of dairy cattle*. 7th Revised edition. National Academy Press: Washington, DC.

Öpik H, Rolfe SA. 2005. *The Physiology of Flowering Plants.* Cambridge University Press

Ortega LI, Alfaro M, Martinez-Lagos J. 2016. Soil nitrogen contribution to grasslands and its implication for nitrogen use efficiency. *Journal of Soil Science and Plant Nutrition* 16: 310–322.

Pan YP, Wang YS, Tang GQ, Wu D. 2012. Wet and dry deposition of atmospheric nitrogen at ten sites in Northern China. *Atmospheric Chemistry and Physics* 12: 6515–6535.

Parfitt RL, Couper J, Parkinson R, Schon NL, Stevenson BA. 2012. Effect of nitrogen fertilizer on nitrogen pools and soil communities under grazed pastures. *New Zealand Journal of Agricultural Research* 55: 217–233.

Pearson CJ, Kempb I, Kirbyc AC, Laundersd TE, Mikled C. 1985. Responsiveness to seasonal temperature and nitrogen among genotypes of kikuyu, paspalum and bermuda grass pastures of coastal New South Wales. *Australian Journal of Experimental Agriculture* 25: 109–116.

Pembleton KG, Rawnsley RP, Burkitt LL. 2013. Environmental influences on optimum nitrogen fertiliser rates for temperate dairy pastures. *European Journal of Agronomy* 45: 132–141.

Piceno YM, Lovell CR. 2000. Stability in natural bacterial communities: I. Nutrient addition effects on rhizosphere diazotroph assemblage composition. *Microbial Ecology* 39: 32–40.

Postgate JR. 1974. New Advances and Future Potential in Biological Nitrogen Fixation. *Journal of Applied Microbiology* 37: 185–202.

Postgate JR. 1998. The origins of the unit of nitrogen fixation at the University of Sussex*. Notes and Records of the Royal Society of London* 52: 355–362.

Qiu H, Ge T, Liu J, Chen X, Hu Y, Wu J, Su Y, Kuzyakov Y. 2018. Effects of biotic and abiotic factors on soil organic matter mineralization: Experiments and structural modelling analysis. *European Journal of Soil Biology* 84: 27–34.

Radhakrishnan M, Waisel Y, Sternberg M. 2006. Kikuyu Grass: A Valuable Salt-Tolerant Fodder Grass. *Communications in Soil Science and Plant Analysis* 37: 1269–1279.

Recous S, Mary B. 1990. Microbial immobilization of ammonium and nitrate in cultivated soils. *Soil*

Biology and Biochemistry 22: 913–922.

Reeves M, Fulkerson WJ, Kellaway RC. 1996. Forage quality of kikuyu (*Pennisetum clandestinum*): The effect of time of defoliation and nitrogen fertiliser application and in comparison with perennial ryegrass (*Lolium perenne*). *Australian Journal of Agricultural Research* 47: 1349–1359.

Rennenberg H, Dannenmann M. 2015. Nitrogen Nutrition of Trees in Temperate Forests—The Significance of Nitrogen Availability in the Pedosphere and Atmosphere. *Forests* 6: 2820–2835.

Rennenberg H, Dannenmann M, Gessler A, Kreuzwieser J, Simon J, Papen H. 2009. Nitrogen balance in forest soils: Nutritional limitation of plants under climate change stresses. *Plant Biology* 11: 4–23.

Robertson GP. Groffman PM. 2015. Nitrogen transformations. In: Paul EA (ed), *Soil microbiology, ecology and biochemistry* (4th edn.). Academic Press, Burlington, Massachusetts, USA. pp 421-446.

Roscoe R, Vasconcellos CA, Furtini-Neto AE, Guedes GAA, Fernandes LA. 2000. Urease activity and its relation to soil organic matter, microbial biomass nitrogen and urea-nitrogen assimilation by maize in a Brazilian Oxisol under no-tillage and tillage systems. *Biology and Fertility of Soils* 32: 52–59.

Ruz-Jerez BE, White RE, Ball PR. 1994. Long-term measurement of denitrification in three contrasting pastures grazed by sheep. *Soil Biology Biochemistry* 26(1):29–39.

Sagi M, Savidov NA, L'vov NP, Lips SH. 1997. Nitrate reductase and molybdenum cofactor in annual ryegrass as affected by salinity and nitrogen source. *Physiologia Plantarum* 99: 546–553.

Schipper A, Schipper L, Hornsby A. 1996. The nitrogen cycle. *The Science Teacher* 63: 34–37.

Scowcroft PG, Haraguchi JE, Hue N V. 2004. Reforestation and Topography Affect Montane Soil Properties, Nitrogen Pools, and Nitrogen Transformations in Hawaii. *Soil Science Society of America Journal* 68: 959.

Shepherd M, Chambers B. 2007. Managing nitrogen on the farm: The devil is in the detail. *Journal of the Science of Food and Agriculture* 87: 558–58.

Silva RG, Cameron KC, Di HJ, Jorgensen EE. 2005. A lysimeter study to investigate the effect of dairy effluent and urea on cattle urine N losses, plant uptake and soil retention. *Water, Air, and Soil Pollution* 164: 57–78.

Sinclair K, Beale PJ. 2010. Critical factors influencing no-till establishment of short-term ryegrass (*Lolium multiflorum*) into a kikuyu (*Pennisetum clandestinum*) pasture. *Crop and Pasture Science* 61: 192–200.

Soussana JF, Lüscher A. 2006. Temperate grasslands and global atmospheric change. *Grassland Science in Europe* 11: 739–748.

Sparling G, Lilburne L, Vojvodic-Vukovic M. 2008. Provisional Targets for Soil Quality Indicators in New Zealand. *Landcare Research Scinece Series No. 34*. Manaaki Whenua Press. Palmerston North, N.Z.

Sprent JI, James EK. 2007. Legume Evolution: Where Do Nodules and Mycorrhizas Fit In? *Plant Physiology* 144: 575–581.

Ste-Marie C, Paré D. 1999. Soil, pH and N availability effects on net nitrification in the forest floors of a range of boreal forest stands. *Soil Biology and Biochemistry* 31: 1579–1589.

Sun X, Luo N, Longhurst B, Luo J. 2008. Fertiliser Nitrogen and Factors Affecting Pasture Responses. *The Open Agriculture Journal* 2: 35–42.

Swanepoel PA, Botha PR, du Preez CC, Snyman HA, Labuschagne J. 2015. Managing cultivated pastures for improving soil quality in South Africa: challenges and opportunities. *African Journal of Range and Forage Science* 32: 91–96.

Swanepoel PA, Habig J, Preez CC, Botha PR, Snyman HA. 2014a. Biological quality of a podzolic soil after 19 years of irrigated minimum-till kikuyu-ryegrass pasture. *Soil Research* 52: 64–75.

Swanepoel PA, Botha PR, Snyman HA, Preez CC. 2014b. Impact of cultivation method on productivity and botanical composition of a kikuyu-ryegrass pasture. *African Journal of Range and Forage Science* 31: 215–220.

Tainton NM. 2000. *Pasture Management in South Africa*. University of Natal Press. Pietermaritzburg, South Africa.

Thangarajan R, Bolan NS, Naidu R, Surapaneni A. 2015. Effects of temperature and amendments on nitrogen mineralization in selected Australian soils. *Environmental Science and Pollution Research* 22: 8843–8854.

Theron JF, van Rensburg WLJ. 1998. The influence of nitrogen and defoliation on production and water-use efficiency of *Lolium multiflorum* cv. Midmar. *African Journal of Range and Forage Science* 15: 35–40.

Thom ER, Prestidge RA. 1996. Use of Italian ryegrass on seasonal dairy farms in northern New

Zealand: 1. Feed production and persistence. *New Zealand Journal of Agricultural Research* 39: 223–236.

Thompson RB, Fillery IRP. 1998. Fate of urea nitrogen in sheep urine applied to soil at different times of the year in the pasture–wheat rotation in south Western Australia. *Australian Journal of Agricultural Research* 49: 495–510.

Tian, G., Z. Cai, J. Cao, and X. Li. 2000. Factors affecting ammonia volatilisation from a rice-wheat rotation system. *Chemosphere* 42:123–129.

Tie X, Zhang R, Brasseur G, Lei W. 2002. Global NO_x Production by Lightning. *Journal of Atmospheric Chemistry* 43: 61–74.

van Breemen N. 2002. Natural organic tendency. *Nature* 415: 381–382.

van der Colf J. 2011. The production potential of Kikuyu (*Pennisetum clandestinum*) pastures over-sown with Ryegrass (*Lolium* spp.). MSc(Agric) thesis, University of Pretoria, Pretoria, South Africa.

van der Colf J, Botha PR, Meeske R, Truter WF. 2015a. Grazing capacity, milk production and milk composition of kikuyu over-sown with annual or perennial ryegrass. *African Journal of Range and Forage Science* 32: 143–151.

van der Colf J, Botha PR, Meeske R, Truter WF. 2015b. Seasonal dry matter production, botanical composition and forage quality of kikuyu over-sown with annual or perennial ryegrass. *African Journal of Range and Forage Science* 32: 133–142.

Venuto BC, Redfearn DD, Pitman WD, Alison MW. 2004. Impact of seeding rate on annual ryegrass performance. *Grass and Forage Science* 59: 8–14.

Vogeler I, Cichota R. 2016. Determination of nitrogen fertiliser requirements in dairy production systems based on early indicators. In: *Efficient use of different sources of nitrogen in agriculture–from theory to practice Skara, Sweden 27 June–29 June 2016: 20.*

Vogeler I, Lucci G, Shepherd M. 2016. An assessment of the effects of fertilizer nitrogen management on nitrate leaching risk from grazed dairy pasture. *Journal of Agricultural Science* 154: 407–424.

Wang C, Wan S, Xing X, Zhang L, Han X. 2006. Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. *Soil Biology and Biochemistry* 38: 1101–1110.

Weigelt A, King R, Bol R, Bardgett RD. 2003. Inter-specific variability in organic nitrogen uptake of three temperate grassland species. *Journal of Plant Nutrition and Soil Science* 166: 606–

611.

Williams MC, Smith BJ, V RL. 1991. Effect of nitrogen, sodium, and potassium on nitrate and oxalate concentration in Kikuyugrass. *Weed Technology* 5: 553–556.

Yerokun OA. 1997. Ammonia volatilization from ammonium nitrate, urea and urea phosphate fertilizers applied to alkaline soils. *South African Journal of Plant and Soil* 14: 67–70.

Zahran HH. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Biology Reviews* 63: 968–989.

Zak DR, Holmes WE, MacDonald NW, Pregitzer KS. 1999. Soil temperature, matric potential, and the kinetics of microbial respiration and nitrogen mineralization. *Soil Science Society of America Journal* 63: 575.

Zhang X, Wang Q, Gilliam FS, Bai W, Han X, Li L. 2012. Effect of nitrogen fertilization on net nitrogen mineralization in a grassland soil, northern China. *Grass and Forage Science* 67: 219–230.

Chapter 3 The effect of N fertilisation on the chemical and biological soil characteristics

3.1 Introduction

In the southern Cape of South Africa, most of the dairy production systems under irrigation are based on planted pastures and commonly consist of kikuyu (*Pennisetum clandestinum*) over-sown with annual ryegrass (*Lolium multiflorum*) (Botha 2003; van der Colf 2011; Swanepoel et al. 2014a). One of the management practices of kikuyu-ryegrass pastures include, high rates of nitrogen (N) fertilisation in the range of 300 to 500 kg N ha^{-1} yr^{-1} (Marais 2001), but these rates are under scrutiny. The motivation behind this, is that the guidelines were developed on cultivated cutting trials (Beyers 1994) without considering N input from grazing animals. These recommendations were thus developed under conditions that are considerably different to grazed, minimum-tillage dairy pastures that are currently adopted by most dairy farmers. It is inevitable that the N dynamics in the soil would also have changed since the development of these N fertiliser guidelines. There is thus a need to reassess the current N fertilisation guidelines for kikuyu and kikuyu-ryegrass systems, in order to find optimum N fertiliser rates under the current management practices (Swanepoel et al. 2015a) which is grazed and associated with minimum-tillage.

Nitrogen dynamics in soil is complex. Besides the addition of N through the excretion of animals and inorganic fertilisers, the soil also supplies N by means of the mineralisation of organic matter (Addiscott 1996). The rate of mineralisation may be affected by both environmental and management factors. Through the process of mineralisation, ammonium is produced with the help of microbes (Edenborn et al. 2011). In soil, ammonium is transformed to nitrite and subsequently nitrate (Brady and Weil 2002). Nitrate is soluble in water, making it vulnerable to potential leaching into the ground water reserves (Addiscott, 1996), which results in pollution and damage to the environment through eutrophication (Conley et al. 2009). In addition, leaching results in the loss of one of the most expensive inputs on a dairy-pasture farm (Baligar et al. 2001). Applying fertiliser increases the risk of N leaching either directly during periods of high rainfall or low pasture production, or indirectly by increasing the urinary N concentration in soil due to increased stocking rates that result from highly productive pasture (Vogeler et al. 2016). Bhogal et al. (2004) found that long term N fertilisation can increase N supply from the soil through mineralisation. This could be associated with an increase in organic carbon due to increase in plant residue returns. Soils under permanent pasture usually have a high content of organic matter and therefore a large pool of potentially mineralisable N (PMN) (Matlou and Haynes 2006). It is therefore important to evaluate the N contribution from mineralisation (Murphy et al. 2004). Since mineralised N is plant available, it should be taken into account when fertilisation decisions are made. For instance lowering fertiliser N applications in times when N from mineralisation are high enough to support productive growth. Organic carbon (C) levels in the soil have also been noted to affect N mineralisation and as such can affect total soil N levels (Swanepoel et al. 2015b). In addition, total N and urease activity have also been found to be highly correlated (Swanepoel et al. 2014c). Similarly, high correlation were also found by Gianfreda et al. (2005) between UA and total N (r=0.886). These studies indicate the importance of the urease enzyme in the N cycle (Pascual et al. 1999) that catalyses the hydrolysis of urea to produce ammonium and carbon dioxide.

In order to effectively reassess the guidelines, soil N dynamics should be investigated as affected by fertilisation and N additions through manure and urine from dairy cows. The aim of this study was to investigate the effects of fertilisation on soil N dynamics, which will aid in optimising N fertilisation of kikuyu and kikuyu-ryegrass pastures. In order to achieve the aim of the study, the effects of different N rates on various soil characteristics such as PMN, C:N ratio, UA and total soil N are determined in order to better understand the N dynamics in the soil as influenced by grazing, N fertiliser input and season. The results may aid in the prevention of losses through leaching while adequate supply to pasture crops are maintained.

3.2 Materials and Methods

3.2.1 Experimental site characterisation

The research site was located on the Outeniqua Research Farm (33 58’ 38” S; 22 25’ 16” E) near George in the Western Cape Province of South Africa. The site is situated at an altitude of 201 m, approximately 8 km from the Indian Ocean. The long-term (35 years) mean annual precipitation is 728 mm and is distributed throughout the year. The area is characterised by a temperate climate with mean daily maximum temperatures in the range of 18 to 25 °C and minimum temperatures of 7 to 15°C. The current study was undertaken for two years and commenced during April 2016 and continued until March 2018. Autumn months were March, April and May; winter months June, July and August; spring September, October and November and the summer months December, January and February. The soil is characterised as a Podzol (Table 3.1) (IUSS Working Group 2015) also locally known as a Witfontein soil form.

3.2.2 Study design and treatments

Two distinctive trials were conducted on separate pasture sites. The first trial consisted of a kikuyu (*Pennisetum clandestinum*) pasture and the second trial of kikuyu over-sown with annual ryegrass (*Lolium multiflorum*). Preceding long-term management of both sites consisted of over-sowing kikuyu pasture with ryegrass employing minimum-tillage methods and an N fertilisation rate of approximately 40 kg N ha^{-1} after each grazing.

Table 3.1:: Description of a Podzolic soil (IUSS Working Group 2015)

Horizon	Depth	Description
Orthic A horizon	0 – 200 mm	Pale grey with low clay content
Podzol B horizon	200 – 300 mm	Accumulation of illuvial organic matter
Unconsolidated material	300 – 600 mm	

The trials were laid out in randomised block designs. Each site had six N fertilisation treatments with four blocks that served as replicates. Plots comprised an area of 15 m × 15 m. Both sites was individually managed and assessed. The six N fertilisation rates were applied to each pasture type in the form of limestone ammonium nitrate (LAN), as soon as possible after grazing, usually within five days of grazing. Five of the six N treatments were applied at fixed rates of 0, 20, 40, 60 and 80 kg N ha^{-1} after each grazing cycle. For the sixth treatment, N was applied strategically, viz. according to the nitrate concentration of the soil water. The fixed nitrogen treatments and criteria utilised in the strategic nitrogen treatment are described in Table 3.2. Soil water was obtained from FullStop^{TM} Wetting Front Detectors (WFD) installed at 150 and 300 mm beneath the soil surface. A WFD is a funnel-shaped passive lysimeter, installed at different depths in soil for the purpose of managing irrigation. When the soil potential around the WFD approaches a matrix potential of c. -3 kPa, free water is produced at the base of the funnel, from which soil water can then be extracted and used to monitor fertilisation and salinity. As the water passes through a filter and collects at the reservoir, a float is magnetically activated showing that there is a water sample to be retrieved for analysis by using a syringe (Stirzaker 2003). During the study the sampled water was moved from the field to the lab in 30 mL watertight containers. Both the syringe and containers were cleaned with distilled water prior to soil water collection. Nitrate concentrations from these soil water samples were determined using a Horiba Scientific LAQUAtwin compact water quality meter. This was done throughout the year whenever the WFD had collected enough water and the float was activated. An average of the preceding month's measurements of the 150 mm WFD was used to calculate the N application rate for the strategic N treatment. When the average nitrate concentration was above 50 mg L^{-1}, no N was applied. At a nitrate concentration between 25 and 50 mg L^{-1} N was applied at a rate of 25 kg N ha^{-1}. When the nitrate concentration was below 25 kg N ha^{-1}, 50 kg N ha^{-1} was applied. According to Fessehazion et al. (2011) the optimum nitrate level in soil solution when both yield and forage quality of ryegrass is considered to be 50 mg L^{-1} . Assuming that mixed pasture systems will behave similarly in the podzolic soil, the optimal nitrate concentration was also set at 50 mg nitrate L^{-1}. However, after September 2016 these recommendations were reconsidered as the strategic N plots maintained soil nitrate levels >50 mg L^{-1}, with evident visual N deficiencies and slow growth. New nitrate concentrations limits were set at >75 mg L^{-1}; 50 to 75 mg L^{-1} and <50 mg L^{-1} to apply 0, 25 and 50 kg N ha^{-1}, respectively.

Table 3.2: The Nitrogen (N) treatments abbreviations, N application rate per grazing cycle and estimated N application rate per year,applied to the kikuyu and kikuyu-ryegrass sites

Abbreviation	N applied after grazing (kg N ha^{-1} grazing cycle^{-1})	Estimated N applied per year (kg N ha^{-1} year^{-1})
N0	0	0
N20	20	200
N40	40	400
N60	60	600
N80	80	800
Nvar	Depend on nitrate concentration in soil solution	

The total amount of N applied varied between the two sites, as seen in Table 3.3. The difference in N application between the two sites will be discussed in the next section (3.2.3).

Table 3.3: Actual total amounts of nitrogen (N) fertiliser applications for year one (1) and two (2) of the study, amount of grazing cycles obtained within a year for the kikuyu and kikuyu-ryegrass site.

	Total application on kikuyu site (kg N ha^{-1} year^{-1})		Total application on kikuyu-ryegrass site (kg N ha^{-1} year^{-1})	
Treatment	Year 1	Year 2	Year 1	Year 2
N0	0	0	0	0
N20	240	260	200	220
N40	480	520	400	440
N60	720	780	600	660
N80	960	1040	800	880
Nvar	35 – 85	0 – 50	10 – 35	0 – 50
Nr of grazing cycles	12	13	11	12
Days in production year	356	379	356	379

N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1}; Nvar = variable rate of nitrogen fertilisation

3.2.3 Pasture management

The kikuyu pasture type was slashed with a mower to a 50 mm height during April 2016 in order to prevent the build-up of a fibrous mat of plant material. No N was applied after slashing in 2016. The kikuyu pasture was slashed again in March 2017, but this year, N treatments were applied after slashing.

For the kikuyu-ryegrass pasture site, the existing kikuyu (local strain) was over-sown with ryegrass (cv. Barmultra II) in April 2016. The existing kikuyu was grazed down to a 50 mm height after which it was mulched to ground level (1.6 meter Nobili with 24 blades). Annual ryegrass was planted into kikuyu at 25 kg ha^{-1} using a minimum-till Aitchison seed drill (van der Colf 2011). The planted area was then rolled with a Cambridge type roller. No N was applied at planting. In March 2017, the same procedure was followed as described above to over-sow ryegrass into kikuyu for the production year starting March 2017. During over-sowing in both years, no N was applied. This was to prevent kikuyu from having a competitive advantage over the newly establishing ryegrass pasture, thereby ensuring that the ryegrass seedlings are not overshadowed by actively growing and fertilised kikuyu. This is also the reason why, in Table 3.3, the kikuyu-ryegrass pasture could accommodate 11 grazing cycles, but the amount of N applied equates to 10 grazing cycles.

Permanent sprinkler irrigation was used on both pastures. Sprinklers were spaced 15 m apart, which also indicated the plot boundaries. Irrigation was based on tensiometer readings, and was aimed at maintaining the matrix potential between -25 and -10 kPa. One tensiometer was installed at a depth of 150 mm on each pasture site. Rain gauges were installed next to the tensiometers to measure the amount of water (rain and/or irrigation) received by the pasture. A Decagon Weather Station was used to collect weather data (Figure 3.1).

The pasture was grazed by Jersey cows, with grazing cycles varying between approximately 28 days during summer and 32 days during winter. A rising plate meter was used to estimate the amount of pasture available for the cows pre-grazing. The plots were strip grazed intensively in order to equally distribute any carry-over effects from either the concentrate from the milking parlour or previous pasture.

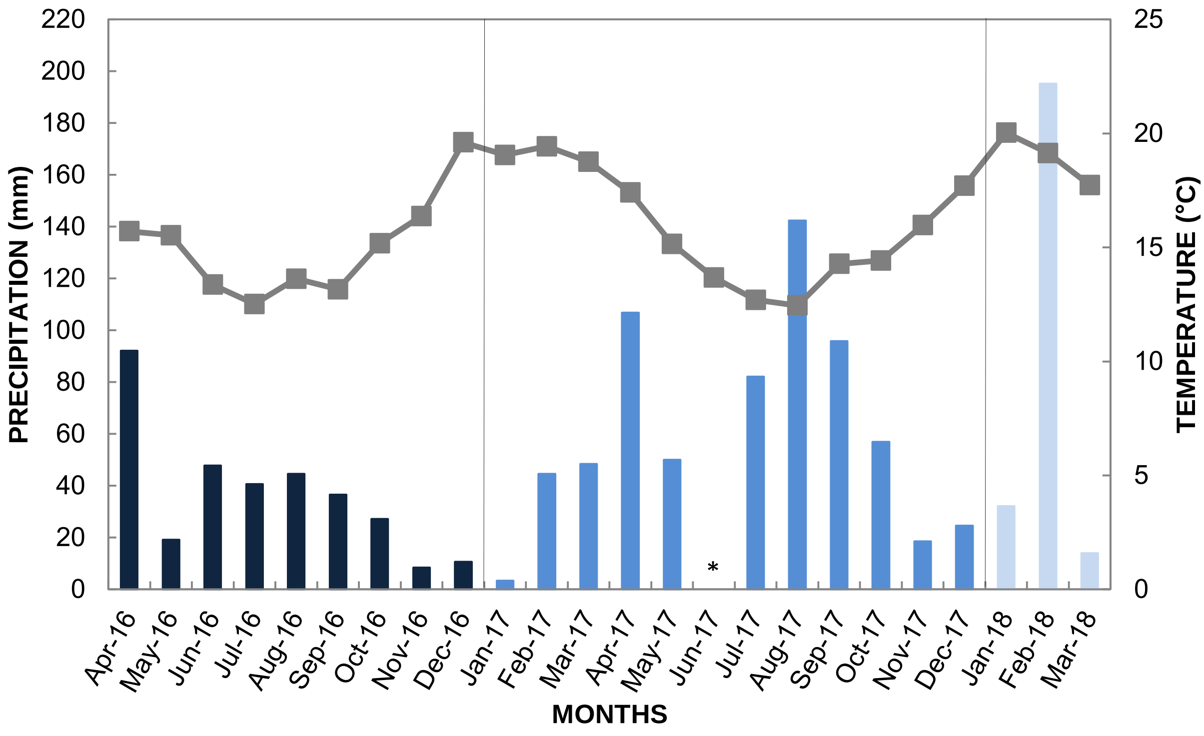

Figure 3.1: Precipitation and mean daily temperature of the Outeniqua Research farm during the course of the study. *faulty instrument, missing value

3.2.4 Sampling and analysis

Soil samples were taken on an annual basis (May 2016 and March 2017) to be analysed for Ca, Mg, Na, K, P, Cu, Zn, Mn, B, S, and C levels in the top 0 – 100 mm depth. The physical and chemical properties prior to N application (May 2016) on both sites are presented in Table 3.4. The soil fertility indicators at both sites were within the recommended guidelines for kikuyu-ryegrass pastures (Beyers 1994), except for certain areas where P, S and B were marginally sub-optimal. To correct these deficiencies, 285 kg ha^{-1} single superphosphate was applied. Solubor (400 kg ha^{-1}) was applied as a foliar spray to alleviate the boron deficiency. In areas where there was free acidity in the soil, 500 kg ha^{-1} calcitic lime was applied. Potassium chloride was applied at a rate according to the soil analysis and K content as per the normal practice.

Table 3.5 and Table 3.6 show the soil analysis results determined during March 2017. These results were obtained after a year of N treatments applications at the kikuyu and kikuyu ryegrass sites, respectively.

Representative soil samples were taken on each plot prior to grazing. Sampling was done in 0 - 100 mm, 100 - 200 mm and 200 - 300 mm depths using a beater type soil auger with a diameter of 75 mm. Three subsamples per plot were taken per depth, pooled and thoroughly mixed in robust plastic basins labelled according to depth. Mixing was done by hand. The augers, basins and hands were cleaned prior to each plot using 70% Ethanol and paper towels. The soil samples were placed in marked paper and plastic bags according to the specific analysis to be done.

Table 3.4: Soil physical and chemical property ranges of the kikuyu and kikuyu-ryegrass sites prior to nitrogen (N) applications in May 2016

	Kikuyu	Kikuyu-ryegrass
pH(KCl)	5.4 - 5.7	5.3 - 5.7
Resistance (Ohm)	480 - 680	690 - 870
Exchangeable acidity (cmol kg^{-1})	0.0 - 0.8	0.0 - 0.8
Exchangeable Ca (mg kg^{-1})	598 - 840	674 - 774
Exchangeable Mg (mg kg^{-1})	118 - 216	188 - 227
Exchangeable Na (mg kg^{-1})	88 - 141	96 - 100
Exchangeable K (mg kg^{-1})	81 - 109	79 - 110
Cation exchange capacity (cmol kg^{-1})	5 - 7	6
Extractable P (mg kg^{-1})	24 - 43	44 - 67
Cu (mg kg^{-1})	0.51 - 10.55	1.31 - 3.76
Zn (mg kg^{-1})	7.93 - 10.30	11.67 - 14.66
Mn (mg kg^{-1})	15.31 - 22.25	15.47 - 18.06
B (mg kg^{-1})	0.34 - 0.42	0.37 - 0.40
S (mg kg^{-1})	8.5 – 12.0	5.0 - 7.0
Organic C (%)	2.18 - 2.69	1.83 - 2.30

Samples, to determine PMN, was taken prior to each grazing to a depth of 100 mm, while the 100 – 200 mm and 200 – 300 mm depth were done on a seasonal basis. Salicylic acid (Cataldo et al. 1975) and indophenol-blue (Keeney et al. 1982) methods were used to analyse for nitrate and ammonium content, respectively. The sum of the nitrate and ammonium contents was the total mineral N content. The soil was aerobically incubated at 20°C and at 75% of field water capacity for 7 days after which N mineralisation was determined (mg kg^{-1}), also using the salicylic acid and indophenol-blue methods. The PMN was presented as stock (kg ha^{-1}). To calculate the stocks, the soil bulk densities of the respective depths were used. Those were 1280, 1519 and 1542 kg m^{-3} for the 0 – 100 mm, 100 – 200 mm and 200 – 300 mm depths, respectively (Swanepoel et al. 2014a).

Table 3.5: Soil physical and chemical property ranges of the kikuyu site after nitrogen (N) applications (soil samples taken during March 2017)

	N0	N20	N40	N60	N80	Nvar
pH(KCl)	5.3	5.4	5.7	5.5	5.6	5.5
Resistance (Ohm)	583	343	555	295	343	553
Exchangeable Acidity (cmol kg^{-1})	0.8	0.8	0.8	0.9	0.9	1.0
Exchangeable Ca (mg kg^{-1})	667	796	852	834	800	824
Exchangeable Mg (mg kg^{-1})	180	210	219	207	202	212
Exchangeable Na (mg kg^{-1})	130	205	141	167	172	157
Exchangeable K (mg kg^{-1})	68	130	64	59	56	59
Cation exchange capacity (cmol kg^{-1})	6	8	7	7	7	7
Extractable P (mg kg^{-1})	68	130	64	59	56	59
Cu (mg kg^{-1})	1.08	1.05	1.39	1.29	1.21	1.12
Zn (mg kg^{-1})	5.65	6.44	8.11	7.39	6.42	6.59
Mn (mg kg^{-1})	7.41	9.79	11.98	10.33	10.74	9.39
B (mg kg^{-1})	0.34	0.37	0.41	0.45	0.39	0.35
S (mg kg^{-1})	8.2	8.8	10.3	9.7	9.7	7.8
Organic C (%)	1.91	2.10	2.16	2.14	2.14	1.75

Table 3.6: Soil physical and chemical property ranges of the kikuyu-ryegrass site after nitrogen (N) applications (soil samples taken during March 2017)

	N0	N20	N40	N60	N80	Nvar
pH(KCl)	5.5	5.5	5.2	5.2	4.9	5.4
Resistance (Ohm)	333	340	358	338	250	305
Exchangeable Acidity (cmol kg^{-1})	0.8	1.0	0.9	1.0	1.1	0.8
Exchangeable Ca (mg kg^{-1})	757	846	766	844	836	797
Exchangeable Mg (mg kg^{-1})	228	248	203	229	216	204
Exchangeable Na (mg kg^{-1})	157	169	139	122	137	181
Exchangeable K (mg kg^{-1})	148	85	69	45	116	100
Cation exchange capacity (cmol kg^{-1})	7	8	7	8	8	7
Extractable P (mg kg^{-1})	53	53	31	44	89	44
Cu (mg kg^{-1})	2.13	2.22	1.92	2.06	2.12	2.00
Zn (mg kg^{-1})	10.72	10.85	9.59	10.80	13.69	9.43
Mn (mg kg^{-1})	13.85	15.46	13.37	15.05	18.19	12.81
B (mg kg^{-1})	0.47	0.50	0.46	0.46	0.50	0.48
S (mg kg^{-1})	8.3	11.5	9.0	7.8	10.4	11.1
Organic C (%)	2.04	2.30	2.18	2.28	2.16	2.07

Urease activity was determined from soil taken at 100 mm depth bimonthly. It was assayed by incubating the soil with urea according to the method of Kandeler and Gerber (1988). These samples were sent, with a wet weight of approximately 75 g, on the day of sampling to the designated laboratory. Enzyme efficiency, as described in Kotzé et al. (2017), was obtained by normalising values. Normalised UA was determined by dividing UA (µg NH_4-N g^{-1} 2 h^{-1}) by the total mineral N (mg kg^{-1}) to express the microbial activity per milligram soil N of normalised values (µg NH_4-N g^{-1} N $2h^{-1}$).

Soil organic carbon was determined through dichromateous digestion utilising the Walkley-Black method (Nelson and Sommers 1982) on samples collected at 100 mm increments to a depth of 300 mm. Ammonium-N was also determined by the Kjeldahl method.

Leco-N content of soil, also referred to as total N in other studies, was determined prior to each grazing at the 0 - 100 mm depth, while 100 - 200 mm and 200 - 300 mm depths were analysed on a seasonal basis through total combustion using a Leco Truspec® CN N analyser (Wright and Bailey 2001). The carbon to N (C:N) ratio of samples were calculated by dividing the Leco-N by the soil organic carbon.

The sample wet weights of PMN and Leco-N were aimed to be approximately 600 g when put into marked paper bags. Both the Leco-N content and PMN samples were weighed on a wet basis and placed in the oven at 40°C for approximately 3 days to be dried. The gravimetric soil water content was determined prior to each grazing. The gravimetric soil water content ranged between 29.6 and 10.1% in the pure kikuyu site, and between 24.1 and 8.4% in the kikuyu-ryegrass pasture in the various depths. It could also be seen that in the 0 – 100 mm depths, the gravimetric soil water content was generally lower than in the 200 – 300 mm depth (Table 3.7). After drying of the soil samples, it was sieved (2 mm) and stored at room temperature until it could be sent to the specific laboratories.

3.2.5 Statistical analyses

The differences between treatments were tested by using the Restricted Maximum Likelihood (REML) method. In order to take the repeated measures into account, the fixed effects were specified as treatment, time (grazing cycle or season) and their interaction. Block was specified as a random effect. The means were separated using Fishers' Protected Least Significant Difference (LSD) test at a 5% significance level (Glass et al. 1972; Snedecor and Cochran 1980). Data was analysed by the STATISTICA version 13.2 (TIBCO Software 2017).

Table 3.7: The average gravimetric soil water content (%) during each sampling for depths 1, 2 and 3 in the pure kikuyu and kikuyu over-sown with annual ryegrass pasture

	Pure kikuyu			Kikuyu annual ryegrass		
	1	2	3	1	2	3
16 May 2016	27.1	19.0	16.5	19.5	13.4	9.7
21 Jun 2016	25.1	17.2	14.9	21.5	15.1	11.3
28 Jul 2016	22.5	17.8	14.3	22.5	14.8	11.5
29 Aug 2016	20.7	14.4	13.0	19.3	13.3	10.8
28 Sep 2016	24.2	16.3	14.6	20.9	14.5	11.9
07 Nov 2016	15.0	11.6	10.1	13.9	10.1	8.4
05 Dec 2016	16.0	11.6	12.8	10.7	10.5	8.5
04 Jan 2017	19.1	14.6	11.8	21.9	12.9	10.6
06 Feb 2017	23.2	16.7	14.3	20.5	13.7	11.1
17 Jul 2017	18.5	13.5	12.5	18.3	13.9	11.3
10 Apr 2017	21.4	14.4	12.4	*	*	*
08 May 2017	22.8	16.5	13.8	18.4	14.9	11.7
12 Jun 2017	29.6	*	*	20.8	*	*
17 Jul 2017	24.7	17.1	14.4	24.1	16.6	13.1
21 Aug 2017	24.2	*	*	22.8	*	*
18 Sept 2017	24.6	*	*	23.1	*	*
24 Oct 2017	18.1	13.0	11.5	14.1	10.9	9.8
20 Nov 2017	21.1	*	*	19.7	*	*
20 Des 2017	20.4	*	*	21.4	*	*
22 Jan 2018	22.9	16.9	14.9	21.4	16.6	13.7
19 Feb 2018	20.6	*	*	20.7	*	*

* sampling did not take place on the specific dates or depths.
1 = 0 – 100 mm depth
2 = 100 – 200 mm depth
3 = 200 – 300 mm depth

3.3 Results and Discussion

3.3.1 Kikuyu pasture site

3.3.1.1 Total mineral nitrogen, nitrate and potential mineralisable nitrogen

The response in terms of total mineral N to treatments varied (P≤0.05) at the 0 – 100 mm depth in all grazing cycles (Table 3.8). The response of total mineral N to treatment in the various grazing cycles can be found in Figure 3.2. During all grazing cycles, N0, N20, N40 and Nvar had similar (P>0.05) total mineral N content when compared within a specific grazing cycle. Treatment N60 and N80 did not follow the same trend. During July and December 2016, and March and July 2017, treatment N80 was higher (P≤0.05) in total mineral N compared to N0. Treatment N60 was higher (P≤0.05) in total mineral N compared to N0 during December 2016, March and July 2017. The increased total mineral N content in treatment N80 and in some instances N60, may indicate that there is a build-up of total mineral N due to the plant's inability to use all the available mineral N. It is therefore necessary to also look at the total mineral N content of the soil in deeper depths, as this can give an indication of whether N is used by plants or lost via leaching.

In Table 3.8 and Figure 3.3, it is evident that the response of total mineral N in the 100 – 200 mm depth also varied (P≤0.05) across grazing cycles and treatments. The soil mineral content was similar (P>0.05) for all treatments within and across grazing cycles up until December 2016. During December 2016, however, treatments N80 and N60 had a higher (P≤0.05) total mineral N compared to treatments N0, N20, N40 and Nvar. This indicates that the pasture produced during November 2016 could not use the total amount of available mineral N in the 0 – 200 mm layer, which likely resulted in a build-up of N in the soil that would have been vulnerable to N leaching.

Table 3.8: ANOVA of the kikuyu site regarding the potential mineralisable nitrogen (PMN) at three soil depths (Num DF=Numerator degrees of freedom, Den. DF= Denominator degrees of freedom)

	Num. DF	Den. DF	F	P-value
Total Mineral N [1] (0 – 100 mm)				
Treatment	5	18	7.43	0.001
Grazing cycle	12	211	2.04	0.023
Treatment* Grazing cycle	60	211	1.71	0.003
Total Mineral N (100 – 200 mm)				
Treatment	5	18	2.02	0.124
Grazing cycle	5	89	0.63	0.678
Treatment* Grazing cycle	25	89	2.42	0.001
Total Mineral N (200 – 300 mm)				
Treatment	5	18	1.82	0.159
Grazing cycle	5	90	21.52	<0.001
Treatment* Grazing cycle	25	90	1.17	0.288
PMN [2] (0 – 100 mm)				
Treatment	5	18	0.43	0.823
Grazing cycle	9	157	6.04	<0.001
Treatment* Grazing cycle	45	157	0.81	0.794
PMN (100 – 200 mm)[3]				
Treatment	5	18	1.18	0.357
Grazing cycle	5	89	4.19	0.002
Treatment* Grazing cycle	25	89	1.32	0.174
PMN (200 – 300 mm)[3]				
Treatment	5	18	1.00	0.445
Grazing cycle	5	90	1.00	0.421
Treatment* Grazing cycle	25	90	1.00	0.475

[1] Total mineral N measured in mg kg^{-1}

() Indicates depth of sampling in soil

* Interaction between main effects

[2] Potential mineralisable N measured in kg ha^{-1} grazing $cycle^{-1}$

[3] Potential mineralisable N measured in kg ha^{-1} $week^{1}$

At a depth of 200 – 300 mm, there were no treatment effects (P>0.05) (Table 3.8). During July 2016 however, the total mineral N in the 200 – 300 mm depth was higher (P≤0.05) compared to the other grazing cycles (Figure 3.4). It might be due high rainfall in June, which could have resulted in a high total mineral N in that depth during July. It can therefore be argued that under a pure kikuyu pasture, at this depth, either most of the mineral N is used successfully by the pasture plants, or that leaching had already occurred. The mean mineral N is 14.9 mg kg^{-1} (Figure 3.4). Total mineral N, comprising of ammonium and nitrate, represents the amount of mineral N that is available to be utilised by the plants. These two components of total mineral N are at risk of being lost to the environment either through leaching, denitrification and volatilisation if not utilised by plants. Fertilisation should therefore not lead to a build-up of mineral N.

Zhang et al. (2012) reported increased mineral N in a study based on a grassland fertilised with 0, 20, 40, 60, 80, 160 kg N ha^{-1} $year^{-1}$ and found significant differences between all treatments. Hatch et al. (2000) found an increase in the soil mineral N when rates of 200 kg N ha^{-1} yr^{-1} were applied, although, the applications were not split equally through the year. Ruz-Jerez et al. (1994) found an increase in soil nitrate from no fertilisation compared to a fertilisation rate of 400 kg N ha^{-1} yr^{-1} on a ryegrass-clover pasture. Even though the current study was on a pure kikuyu pasture, the results are comparable to that of Ruz-Jerez et al. (1994).

Figure 3.5 shows the average nitrate concentration in the soil water on the kikuyu site, at both the 150 and 300 mm depths. The nitrate concentration was always higher than the 50 mg nitrate L^{-1} limit as determined by the European Union (EU) Nitrates Directive (EEC 1991) or the 5 mg L^{-1} of nitrate-N advisable for drinking water in South Africa (DWAF 1996). The nitrate concentration was higher than the aforementioned standards, even during grazing cycles where no N was applied. In a study conducted by Fessehazion et al. (2011) on Cedara in KwaZulu-Natal where WFD's were utilised on a kikuyu and ryegrass pasture, the authors were able to reduce N input without compromising pasture herbage production based on maintaining a soil nitrate concentration of 50 mg nitrate L^{-1}. However, this strategy was not successful in the current study, since the nitrate concentration remained higher than the recommended concentration of 50 mg L^{-1} throughout the study period. A possible reason for the discrepancy in results between this study and that of Fessehazion et al. (2011) might be due to the difference in soil type. The Cedara soil type was a deep (1 m) red, kaolinitic Hutton soil type, while the current study site is characterised by a much shallower (<1 m), sandy loam soil, witfontein soil type. This may also be why the floats were not often latched (and only intermittent soil water collected), as it has been noted that the WFD does not register slow draining rates (Fessehazion et al. 2011). For the 300 mm depth WFD, nitrate concentrations of above 75 mg nitrate L^{-1} were recorded which, since most of the root accumulation is limited to this depth, can be assumed to be vulnerable to leaching (Fessehazion et al. 2012). To make the use of WFD plausible, by getting more readings, irrigation should be adjusted. This however is not a viable option. In addition, preventing grazing cows from damaging the WFD might be daunting. On the current study, wired cages (steal frame with wire) around the WFD were in some instances not enough to keep curious cows away. It is not ideal to often replace a WFD in a pasture system. It causes soil disturbance in minimum-tillage system which will not yield accurate nitrate concentration results.

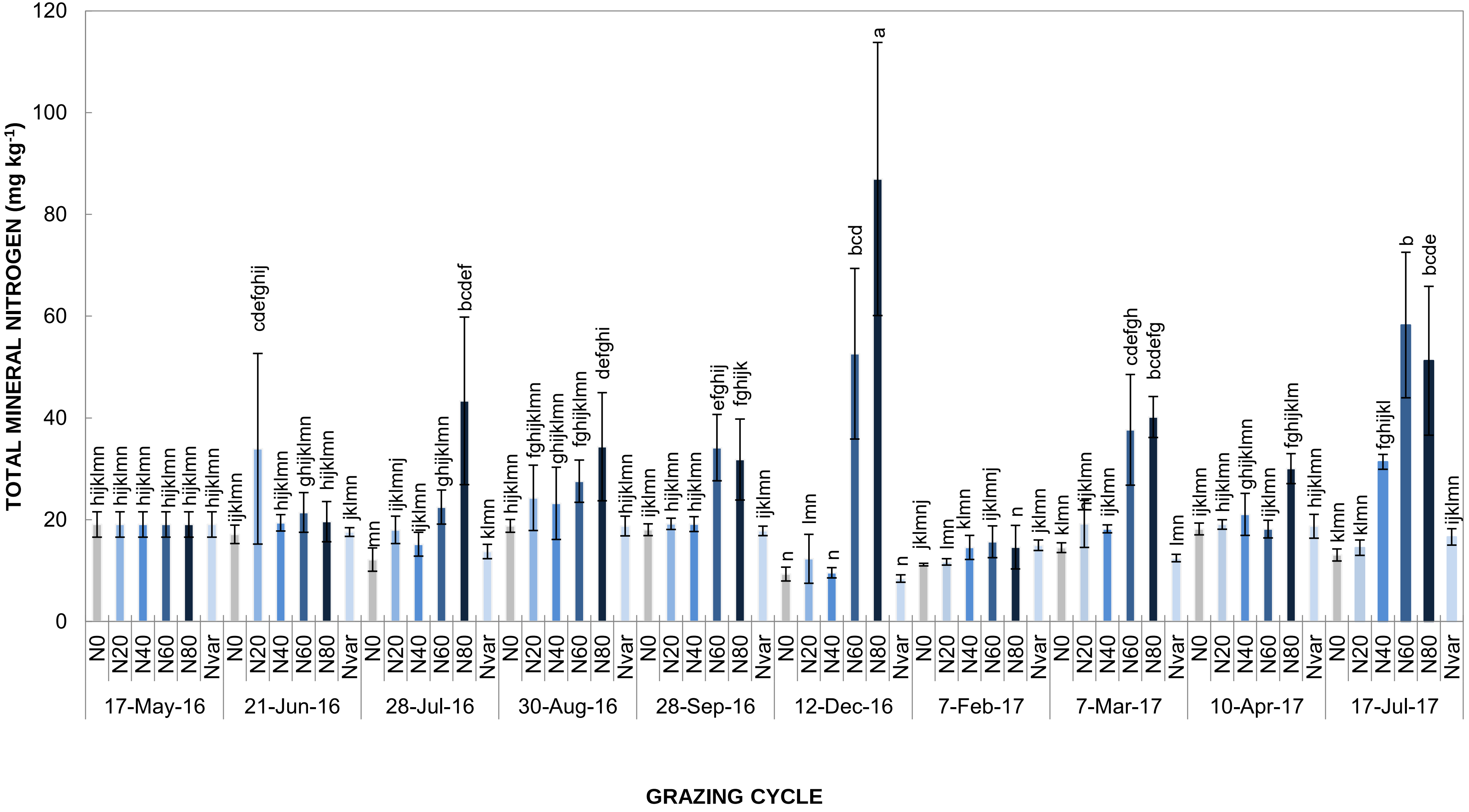

Figure 3.2: Total mineral N (mg kg^{-1}) on the day of sampling at the 0 - 100 mm soil depth on the kikuyu site, affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

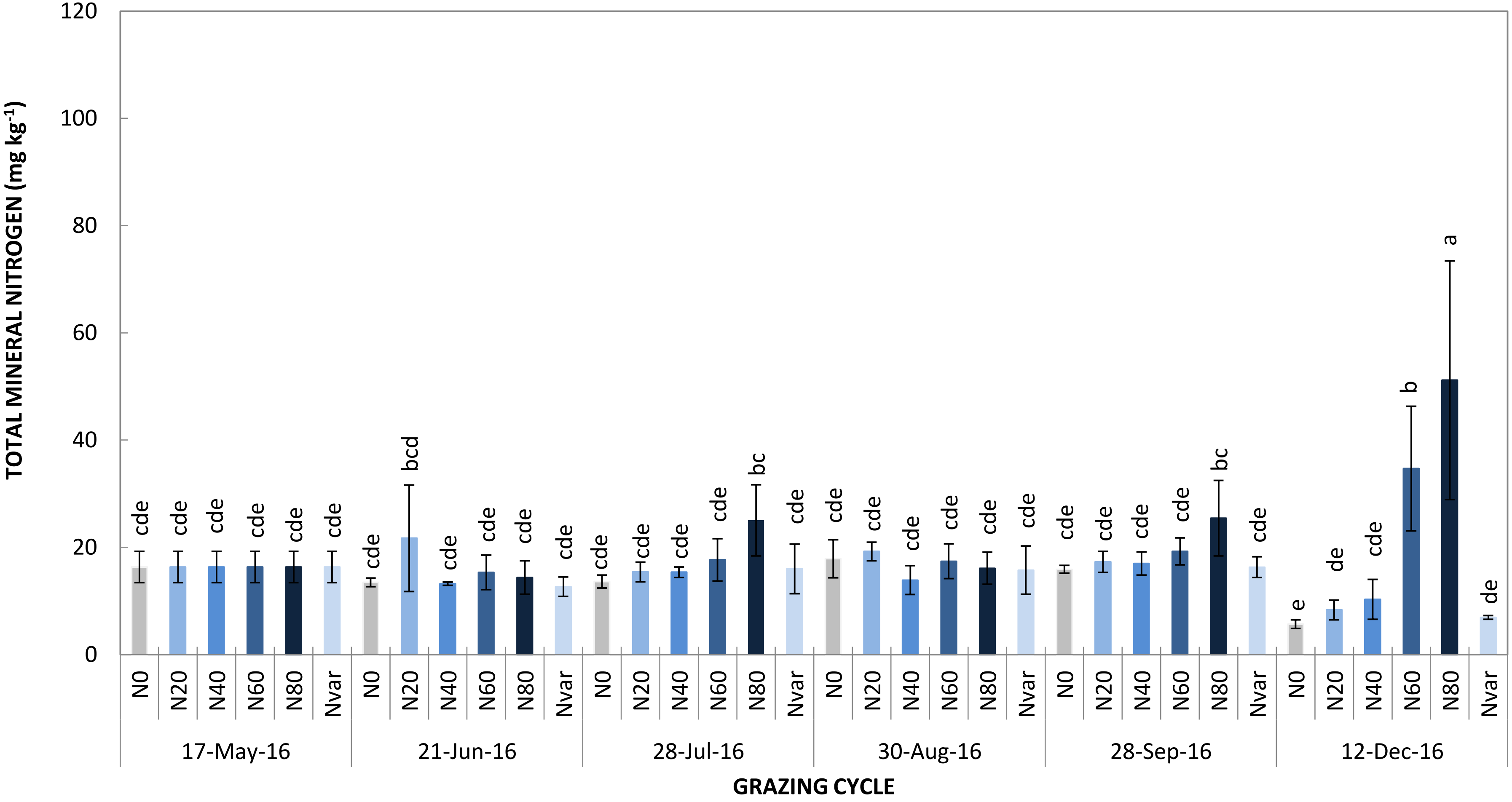

Figure 3.3: Total mineral N (mg kg^{-1}) on day of sampling at the 100 – 200 mm depth, in the kikuyu site, affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

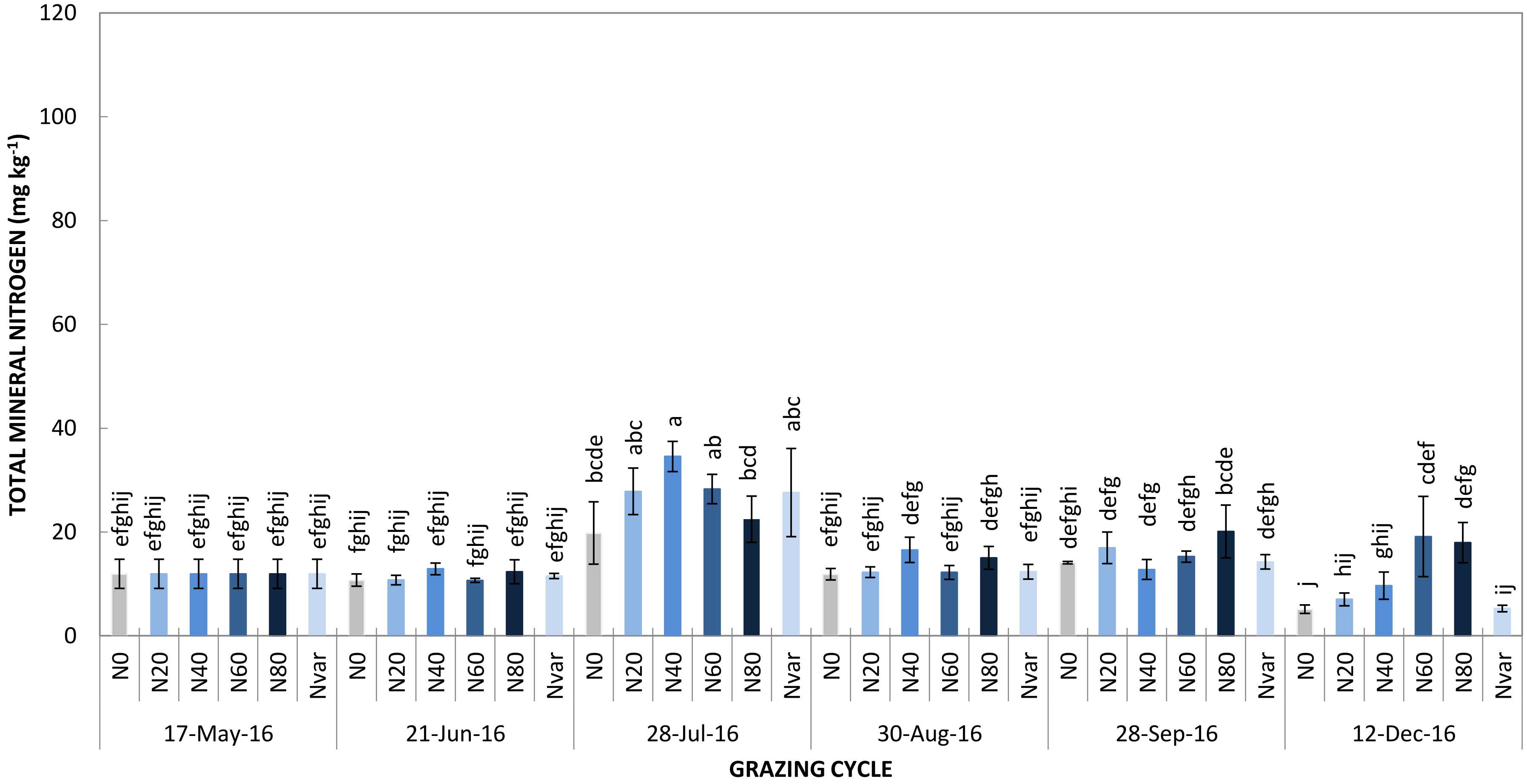

Figure 3.4: Total mineral N (mg kg^{-1}) on day of sampling at the 200 – 300 mm depth in the kikuyu site, affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

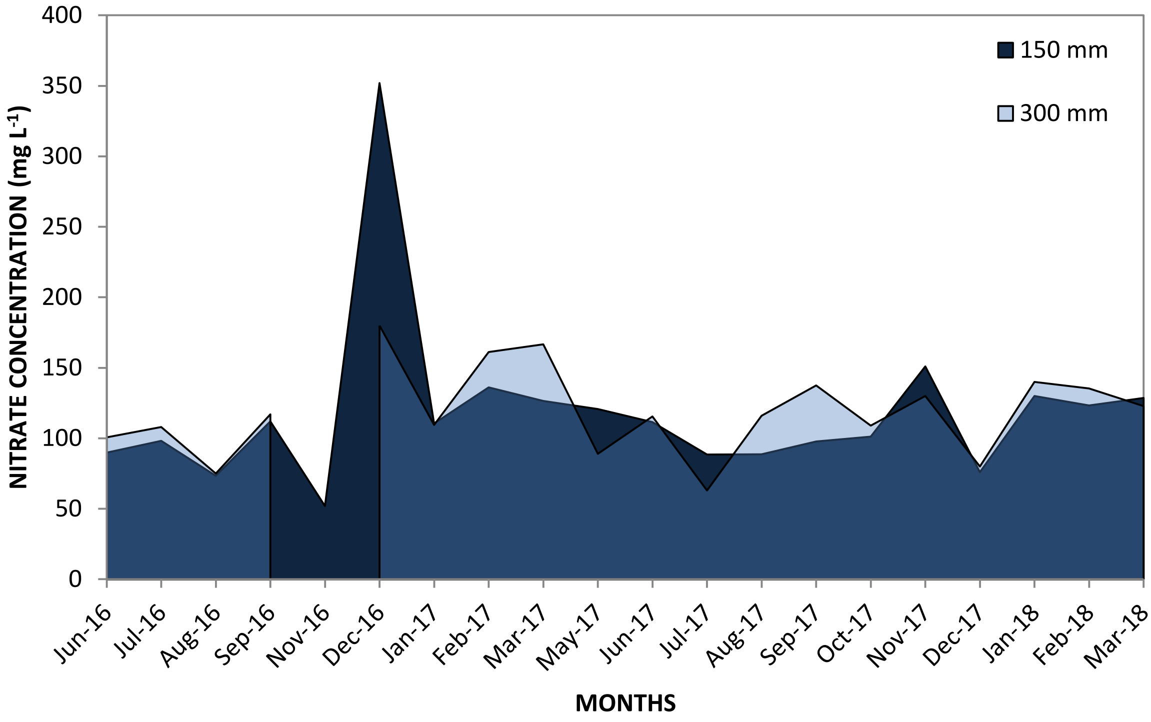

Figure 3.5: Average nitrate concentration (mg nitrate L^{-1}) in soil water collected from wetting front detectors (WFD) for the duration of the trial on the kikuyu site. WFD were used in Nvar plots only, and installed at 150 mm and 300 mm depth in the soil

In Table 3.8, the N treatments did not affect the PMN (P>0.05) between grazing cycles in the 0 – 100 mm depth. For this reason, in Figure 3.6, the results of treatments are combined to illustrate the effect of grazing cycles. The lowest potential to mineralise N in the soil was recorded in July 2016 and March 2017 (P≤0.05) with April 2017 similarly low to July and March (P>0.05). In the 100 – 200 and 200 – 300 mm depths, regarding PMN, there were no interaction (P>0.05) between treatments and grazing cycles (Table 3.8) (data not shown).

The results from current study were irregular through time, so it was not possible to identify clear seasonal trends for the occurrence of the highest PMN. In Australia, it was found that on a seasonal basis PMN was highest during winter months, with the highest PMN values apparent at the high N rate during winter (Pembleton et al. 2013). In the current study, even though PMN values similar to the highest value were recorded in June 2016, August 2016 and July 2017 (grazing cycles within winter), a PMN value similar to the lowest was also recorded during July 2016 (winter). In a wheat production system, higher PMN was also observed during winter at sowing, compared to summer at harvest and it was concluded that PMN was negatively correlated (r = -0.19) with soil temperature (Cookson et al. 2006). The authors also found that PMN was positively correlated (r = 0.17) with soil moisture during the study. These findings were in contrast to those of a study on grassland in China, where a positive correlation of PMN to both soil temperature (r = 0.46) and precipitation (r = 0.5) was found in the 0 – 100 mm soil depth (Zhang et al. 2012). A laboratory study reported that mineralisation rates in China were influenced by incubation temperature, but only at higher temperatures of 15 to 35°C, compared to lower temperatures of -10 to 5°C (Wang et al. 2006). Another study under controlled conditions confirmed that mineralisation rates increased when temperature increased from 25 to 35°C (Guntiñas et al. 2012). They also found that mineralisation rates were higher at higher moisture levels. Dessureault-Rompré et al. (2010), however, found a negative correlation between PMN and annual precipitation. Results in terms of the impact of temperature and soil moisture on PMN thus varied substantially between studies found in the literature.

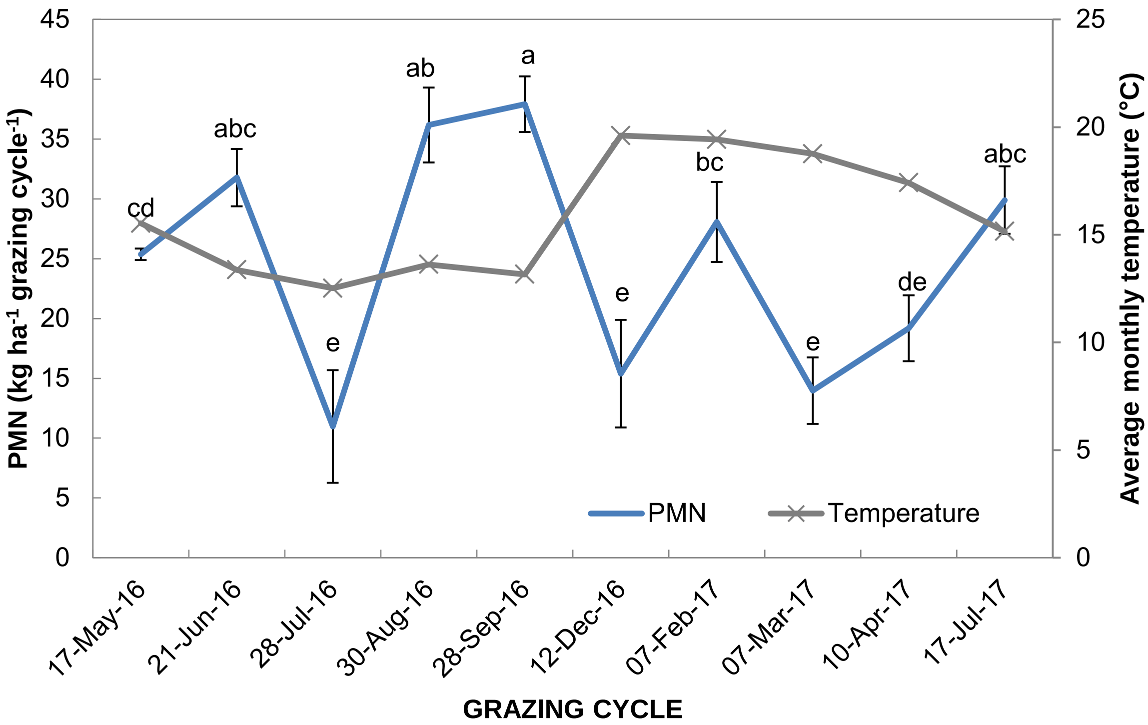

Figure 3.6: Average potential mineralisable nitrogen (PMN) (kg N ha^{-1} grazing cycle^{-1}), averaged over all treatments, at the 0 – 100 mm depth on the kikuyu site of as affected by grazing cycle. Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level. The average monthly temperatures (°C) are also displayed

The average PMN for the current study was 18.8, 21.3, 18.8, 16.7, 16.0 and 21.7 mg kg^{-1} week^{-1} for treatments N0, N20, N40, N60, N80 and Nvar, respectively. The PMN values of a study in the southern Cape region was in the range of -18.2 to 44.4 mg N kg^{-1} week^{-1} while the mean was 10.64 mg N kg^{-1} week^{-1} when approximately 32.0 kg N ha^{-1} after every grazing was applied (Swanepoel et al. 2014c). The results of the current study are therefore slightly higher compared to that of Swanepoel et al. (2014c).

Pasture receiving fertiliser, had higher mineralisation rates compared to unfertilised pasture (Hatch et al. 2000). Linear responses of N mineralisation rates were found up to fertilisation rates of 80 kg ha^{-1} year^{-1} and reported that rates in excess of this may lead to soils that are N saturated (Zhang et al. 2012). The results of the current study contradict what is found in the aforementioned studies.

Since treatment did not have an effect on the PMN of the soil, this is also an indication of N build-up in the soil. The conclusion is that the kikuyu pasture is likely to be N saturated and that N fertiliser application of more than 40 kg N ha^{-1} grazing $cycle^{-1}$ will result in potential leaching losses.

3.3.1.2 Carbon to nitrogen ratio

There was no response (P>0.05) to treatments in terms of C:N ratio within the grazing cycles in the 0 – 100 mm depth (Table 3.9). Although treatments did not have an effect (P>0.05), the grazing cycle affected the C:N ratio (P≤0.05), with the highest ratio occurring during May 2016 followed by June 2016 (P≤0.05) and the lowest (P≤0.05) ratio during July 2016 (Figure 3.7). The baseline C:N values were 23.2 before treatments were applied and then decreased to a ratio of 9.67 in July 2016, and remained below a ratio of 15 throughout the study.

Table 3.9: ANOVA table of the kikuyu site regarding carbon:nitrogen (C:N) ratio in the various depths (Num DF = Numerator degrees of freedom, Den. DF = Denominator degrees of freedom)

	Num. DF	Den. DF	F	P-value
C:N ratio (0 – 100 mm)				
Treatment	5	18	0.45	0.809
Grazing cycle	7	126	181.63	<0.001
Treatment* Grazing cycle	35	126	0.69	0.900
C:N (100 – 200 mm)				
Treatment	5	18	1.46	0.250
Grazing cycle	2	36	142.41	<0.001
Treatment* Grazing cycle	10	36	0.88	0.563
C:N (200 – 300 mm)				
Treatment	5	18	0.79	0.571
Grazing cycle	2	35	5.21	0.010
Treatment* Grazing cycle	10	35	0.81	0.617

() Indicates depth
* Interaction between main effects

As the C content of a soil increase, so does the ability of the soil to store N. However, this trend only occurs up to a certain point, referred to as the steady state, which is dependent on the soil mineralogy, climate and management (Schipper et al. 2004; Dolan et al. 2006). As the soil reaches a steady state of C and is no longer able to store additional N in immobilised forms, a greater degree of N leaching and emissions can be expected (Schipper et al. 2004). The optimal C:N ratio for pasture soil is 8 – 12. This was confirmed by Tainton (2000) who stated that well managed pasture soil has a C:N ratio of 10. A very high ratio (>40) can be viewed as indicative of a N deficiency in the system, while a very low ratio (<5) could indicate a risk of low environmental quality resulting from leaching (Sparling et al. 2008). According to these standards, pasture in the current study may still be able to store additional N. In a study based on the global soil database, Podzols soil (0 – 300 mm depth) are reported to have an average of C:N ratio of 23.8 (Batjes 1996). The values reported in this study are well below this value.

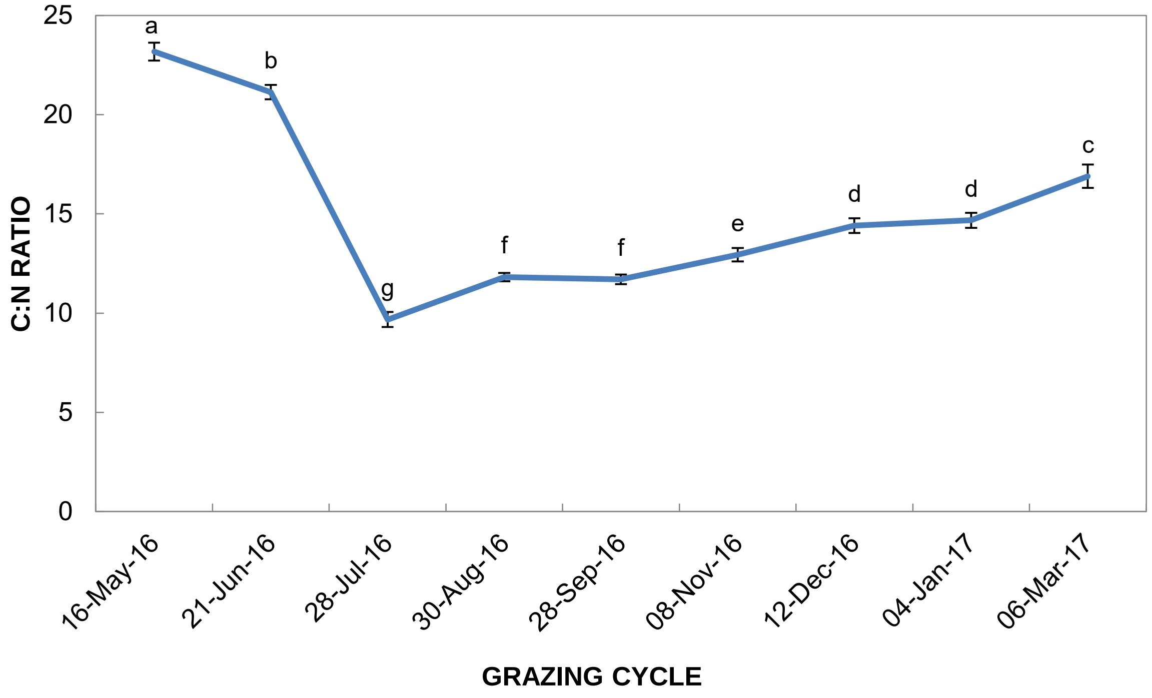

Figure 3.7: Average carbon to nitrogen (C:N) ratio at the 0 – 100 mm depth on the kikuyu site as affected by grazing cycle and averaged over all treatments. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

Miles (1997) found that a productive kikuyu pasture, fertilised with 250 – 350 kg N ha^{-1} $year^{-1}$, had a C:N ratio of 11.7, while unproductive kikuyu sites, not fertilised with N for five years of longer had a C:N ratio of 12.2 and 12.3. Similarly, Swanepoel et al. (2014b) reported C:N values of 11.1 in kikuyu-ryegrass pastures in the southern Cape fertilised with approximately 350 kg N ha^{-1} yr^{-1}. The C:N ratio in the current study were higher compared to the aforementioned studies.

Different substrates have different C:N ratios and may thus impact the C:N ratio differently when applied to a pasture. Substrates with a high C:N ratio are viewed to be of low quality, while a low C:N ratio substrate is viewed as high quality (Hart et al. 1994). Fresh dung in a study conducted in England had a C:N ratio of 13.1 (Hatch et al. 2000). This explains why the C:N ratio was found to be lower under grazing compared to hay producing conditions (Franzluebbers et al. 2000). This is likely due to nutrient return from grazing animals through excreta, while haying also removes the litter and thus nutrients which could have potentially decomposed.

A 50-year fertilisation study on corn in the United States of America, showed a loss of soil organic C, therefore a strategy was proposed to fertilise according to the soil tests rather than yield based, so as to prevent excessive N from accumulating in the soil and reduce oxidation of soil organic carbon by microbes (Khan et al. 2007). In a study over 12 years with the grazing of tall fescue, the C:N ratio in the 150 mm depth also decreased from 16.9 at the beginning of the trial to 15.1 after five years and to 9.4 after 12 years (Franzluebbers and Stuedemann 2009). This might have been due to loss of C or an increase in N. It is therefore important to find the optimal N fertilisation rate in order to prevent the loss of carbon through over fertilisation with N.

3.3.1.3 Urease activity

Grazing cycle and treatment did not show an interaction ($P>0.05$), but grazing cycle affected the UA ($P\leq0.05$) (Table 3.10). There was a decrease ($P\leq0.05$) in all treatments in terms of UA from July 2016 to July 2017, with the exception of Nvar (Figure 3.8). Within this period, treatment N80 declined the most; showing a consistent decrease ($P\leq0.05$) with each sampling date until December 2016 ($P\leq0.05$). Thereafter, treatment N80 increased ($P\leq0.05$) from December 2016 to February 2017 and then stabilising ($P>0.05$) until July 2017. In the same location, UA was found to be 538 µg NH_4-N g^{-1} 2 h^{-1} in the 0 - 100 mm depth (Swanepoel et al. 2014b), which is much higher than the current study's average of 253 µg NH_4-N g^{-1} 2 h^{-1}.

Table 3.10: ANOVA of the kikuyu site regarding urease activity in the 0 – 100 mm depth (Num DF = Numerator degrees of freedom, Den. DF = Denominator degrees of freedom)

	Num. DF	Den. DF	F	P-value
Urease activity[1]				
Treatment	5	15	0.48	0.787
Grazing cycle	5	90	14.01	<0.001
Treatment* Grazing cycle	25	90	1.01	0.459
Urease activity (normalised)[2]				
Treatment	3	49	9.66	<0.001
Grazing cycle	5	18	4.56	0.007
Treatment* Grazing cycle	15	49	3.43	0.001

[1] measured in µg NH_4-N g^{-1} $2h^{-1}$

[2] measured in µg NH_4-N g^{-1} N $2h^{-1}$

() Interaction between main effect

In the Chilean andisols, UA increased as temperature increased (Cartes et al. 2009). A review of UA, presented information that increased activity is observed at increasing temperatures, increased soil moisture and lower C:N ratio (Adetunji et al. 2017).

A greenhouse study in China found that UA were similar in the unfertilised (control) and inorganically fertilised plots, with both lower than, when compared to, composted (varied from plant residues to pig manure) fertilised plots. The authors reported that this was due to the presence of inorganic N forms, it was not to essential synthesise urease (Chang et al. 2007). Several other studies found similar results. For example, in a maize and beans rotation study, there was no difference in UA due to urea application treatments (Roscoe et al. 2000). In another study, UA was increased with the application of slurry from cattle fed either perennial ryegrass or maize silage (Bol et al. 2003). The findings in the current study, where N fertilisation rates did not affect urease activity, are thus similar to previous studies.

Urease activity in clay grassland soil in South Africa under rotational grazing had the highest UA (115 µg NH_4-N g^{-1} soil 2 h^{-1}) under poor grazing conditions and the lowest UA (30 µg NH_4-N g^{-1} soil 2 h^{-1}) under good grazing conditions (Kotzé et al. 2017). However, UA in grassland soil might not be the same as in pasture soil that is intensively managed for good quality pasture. A study conducted in the same region as the current study found that UA was higher in soils under cultivated pasture than in virgin soil in the 0 – 100 mm, 100 – 200 mm and 200 – 300 mm depths (Swanepoel et al. 2014b). The authors also found strong correlation (r = 0.95) between total N and urease. For this reason the UA results of the current study was normalised with total mineral soil N.

When the UA activity data were normalised with total mineral N, different results were found compared to UA data that were not normalised. The response of treatments normalised UA was different ($P \leq 0.05$) in the various grazing cycles as found in the ANOVA Table 3.10. There is a general trend that N80 and N60 had a lower normalised UA, however this was not always significant ($P > 0.05$) (Figure 3.9). This supports the postulation that the urease enzyme is redundant in the presence of mineral forms of N. This is similar to multiple authors that have reported a higher UA in control treatments (no amendments) (Saha et al. 2008) or when high amounts of organic material have been added to the soil (Pascual et al. 1999; Zaman et al. 2002) compared to chemically fertilised plots (Liu et al. 2010).

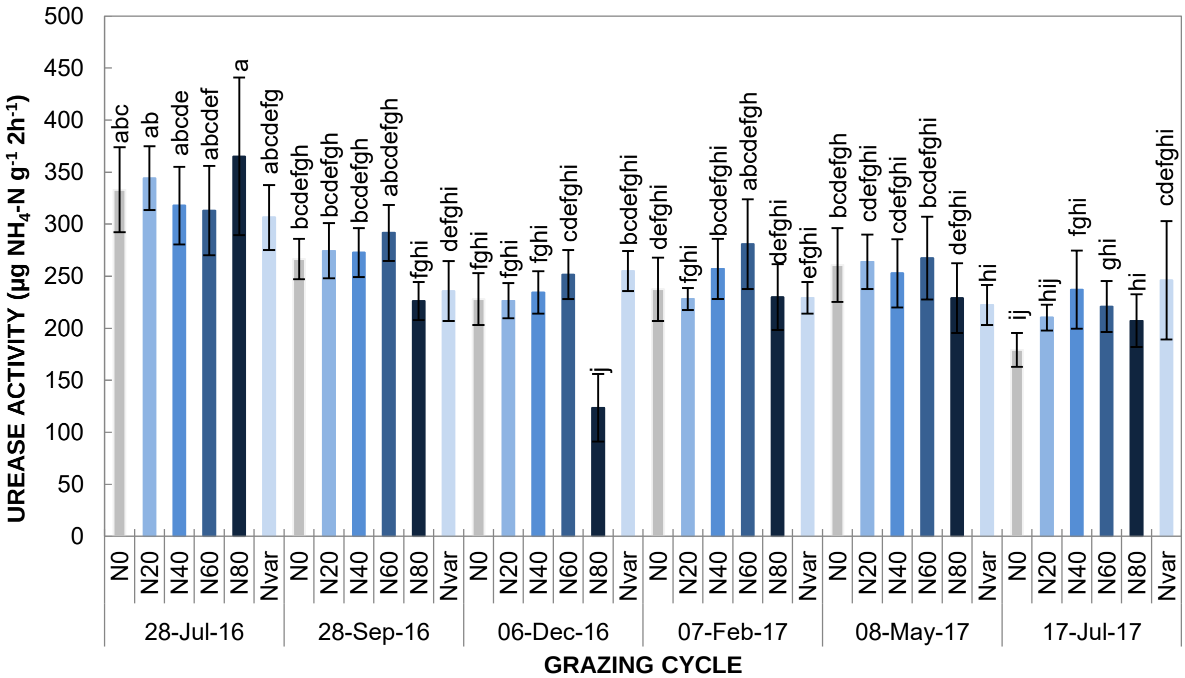

Figure 3.8: Urease activity (µg NH_4-N g^{-1} $2h^{-1}$) at the 0 - 100 mm soil depth on the kikuyu site approximately every second grazing cycle affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

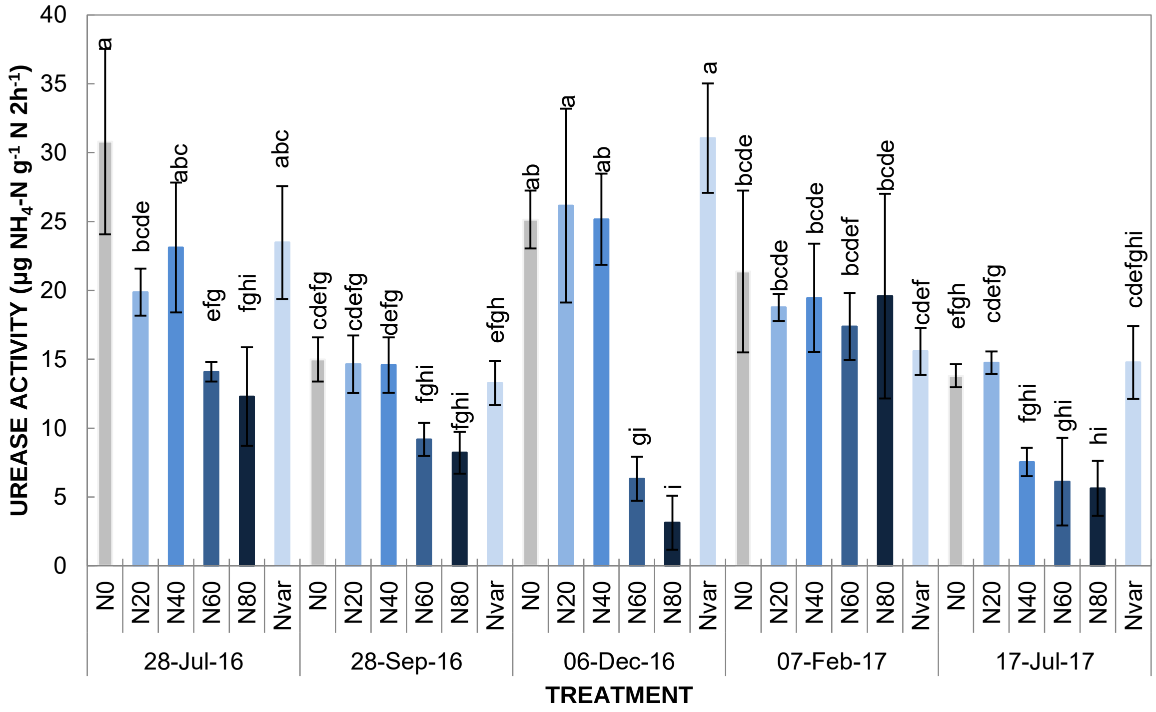

Figure 3.9: Normalised Urease Activity (µg NH_4-N g^{-1} N $2h^{-1}$) in the 0 - 100 mm soil depth on the kikuyu site approximately every second grazing cycle as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

3.3.1.4 Soil Leco-N and Kjeldahl-N

No interaction (P>0.05) between treatment and grazing cycle was found to influence the Kjeldahl-N in the 0 – 100 mm depth (Table 3.11). Kjeldahl-N was however affected by grazing cycle (P≤0.05), with August and September 2016 having a higher (P≤0.05) Kjeldahl-N compared to the other months (Figure 3.14). Therefore, with the exception of September, Kjeldahl N was higher (P≤0.05) in spring months than during winter, summer and autumn. This is similar to what was seen in the Leco-N at this 0 – 100 mm depth. Only grazing cycle influenced (P≤0.05) the Leco-N, not the N treatments (P>0.05; Table 3.12).

A site that was comprised of kikuyu for more than 50 years was sampled and a Kjeldahl-N content of 0.8% was obtained (Matlou and Haynes 2006). This is higher than the 0.22% maximum for the current study site. Swanepoel et al. (2014c) found Kjeldahl-N in the southern Cape region to be an average of 0.34% for kikuyu and ryegrass pastures which is also higher than that was found in the current study. Franzluebbers and Stuedemann (2009) indicated total soil N was higher in grazed pastures compared to pasture not grazed. There is limited information available in literature on total soil N analysed with Leco method on pasture fertilisation. Total soil N work in literature using Leco method is mostly based on wheat (Cookson et al. 2006; Sharifi et al. 2007) and it is thus difficult to determine whether the mean Leco-N value of 0.15% found in the current study was low, average or high for pasture soils.

Table 3.11: ANOVA table of kikuyu site regarding Kjeldahl-Nitrogen (N) in the various depths (Num DF = Numerator degrees of freedom, Den. DF = Denominator degrees of freedom)

	Num. DF	Den. DF	F	P-value
Kjeldahl-N (0 – 100 mm)[1]				
Treatment	5	18	1.40	0.269
Grazing cycle	8	144	11.87	<0.001
Treatment*grazing cycle	40	144	1.06	0.393
Kjeldahl-N (100 – 200 mm)				
Treatment	5	18	1.08	0.404
Grazing cycle	8	144	4.65	<0.001
Treatment*grazing cycle	40	144	0.80	0.794
Kjeldahl-N (200 – 300 mm)				
Treatment	5	18	0.52	0.760
Grazing cycle	8	144	8.59	<0.001
Treatment*grazing cycle	40	144	0.72	0.867

[1] measured as %
() indicates depth
*Interaction between main effects

Table 3.12: ANOVA table of the kikuyu site regarding Leco-Nitrogen (N) in the various depths (Num DF = Numerator degrees of freedom, Den. DF = Denominator degrees of freedom)

	Num. DF	Den. DF	F	P-value
Leco-N (0 – 100 mm)[1]				
Treatment	5	18	2.52	0.067
Grazing cycle	12	216	114.26	<0.001
Treatment*grazing cycle	60	216	0.86	0.748
Leco-N (0 – 100 mm)				
Treatment	5	18	2.07	0.133
Season	3	54	121.56	<0.001
Treatment*Season	15	54	0.99	0.179
Leco-N (100 – 200 mm)				
Treatment	5	18	1.96	0.133
Season	3	54	87.70	<0.001
Treatment*Season	15	54	1.41	0.179
Leco-N (200 – 300 mm)				
Treatment	5	18	1.41	0.268
Season	3	54	31.74	<0.001
Treatment*Season	15	54	0.84	0.627

[1] measured as %
() indicates depth
*Interaction between main effects

Results of year one showed a substantial increase in the Leco-N (total soil N) from June to July 2016, reaching a high in July (P≤0.05). During July treatment N40 had a higher Leco-N than all the other treatments (P≤0.05) (data not shown). A gradual decrease with months from July 2016 to February 2017 is observed (Figure 3.10).

Seasonal data regarding Leco-N at all depths, 0 – 100, 100 – 200, and 200 – 300 mm, showed that only Leco-N was affected (P≤0.05) by season and not by fertiliser treatments (Table 3.12). In the 0 – 100 mm depth, a decrease was observed from winter to spring (P≤0.05), spring to summer (P≤0.05) with summer and autumn being similar (P>0.05) (Figure 3.11). In the 100 – 200 mm depth, Leco-N decreased (P≤0.05) from winter to summer and then remained similar (P>0.05) to autumn. During winter treatment N40, N60 and N80 had a higher Leco-N compared to N20 and Nvar (P≤0.05) (Figure 3.12). In spring treatment N40 had a Leco-N compared to N0 and Nvar (P≤0.05). There were no treatment differences in summer and autumn (P>0.05). Leco-N in the 200 – 300 mm depth remained constant (P>0.05) from winter to spring while the Leco-N decreased from spring to summer (P≤0.05) (Figure 3.13). During winter N40, N60 and N80 was higher in Leco-N compared to Nvar (P≤0.05). It might be that during spring and summer, more N is being removed from this deeper layer due to pasture growth. From summer to autumn there is an increase in the Leco-N the 200 – 300 mm depth (P≤0.05) which might be due to less N being removed from this layer of soil.

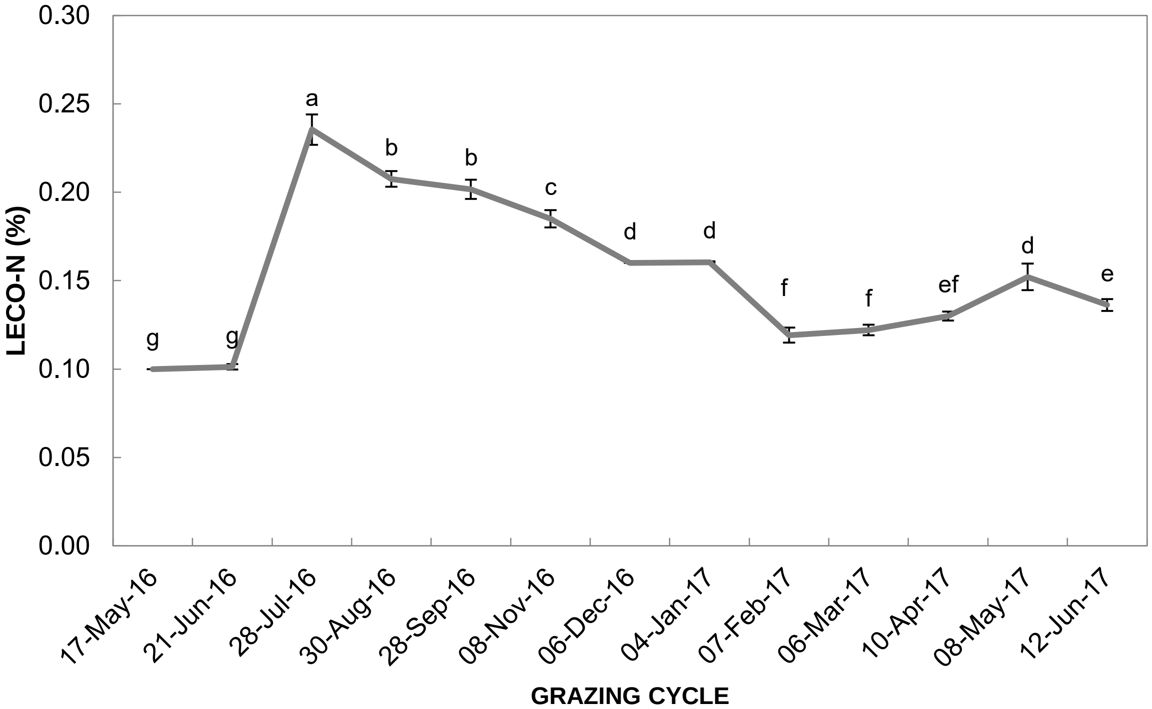

Figure 3.10: Average Leco nitrogen (N) (%) at the 0 – 100 mm depth on the kikuyu site, when averaged over all treatments. Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level

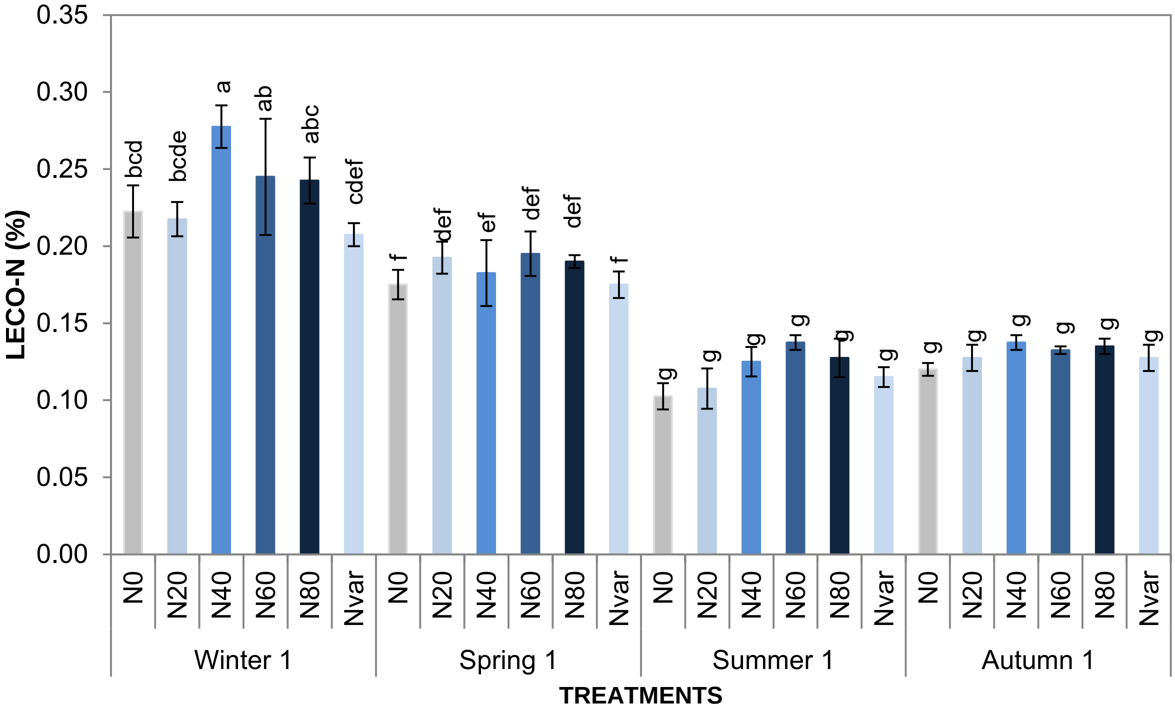

Figure 3.11: Leco nitrogen (N) (%) at the 0 – 100 mm depth in seasons of year one (1) on the kikuyu site, affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

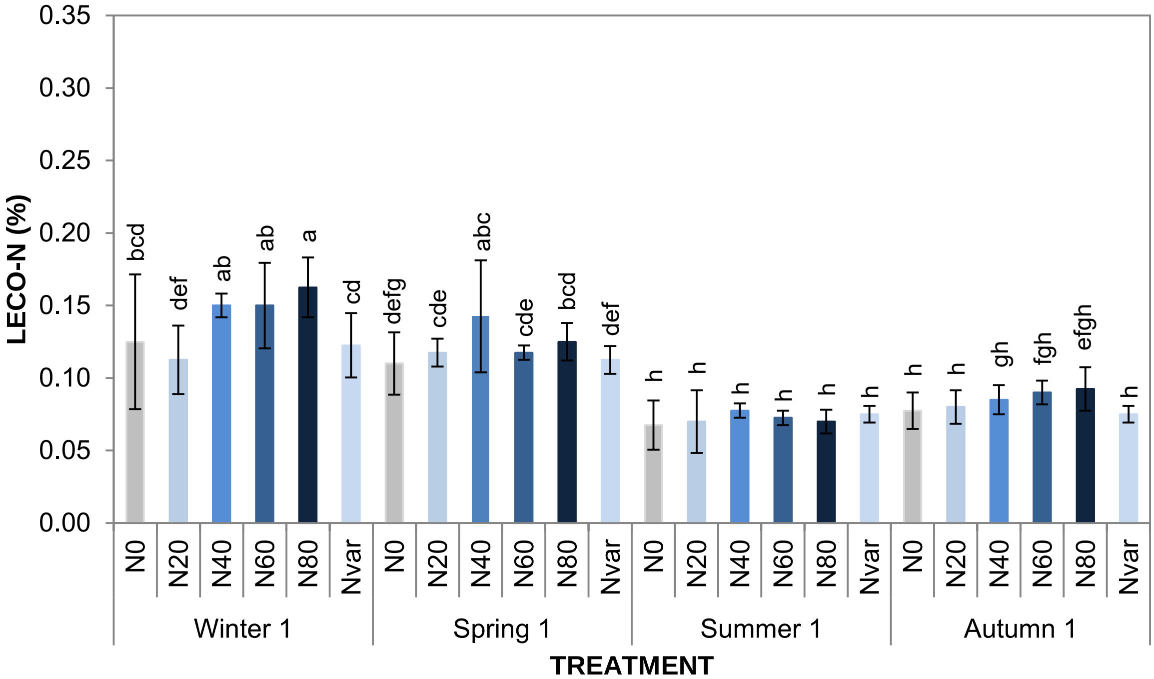

Figure 3.12: Leco nitrogen (N) (%) at the 100 – 200 mm depth in seasons of year one (1) on the kikuyu site, affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

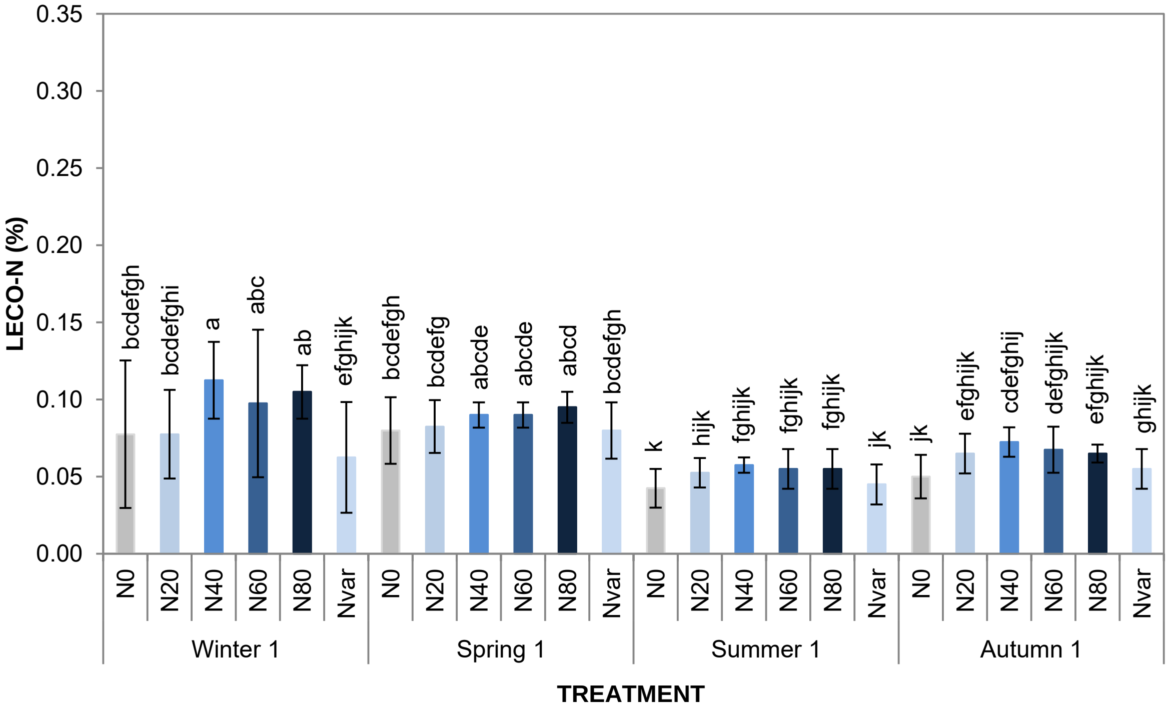

Figure 3.13: Leco nitrogen (N) (%) at the 200 – 300 mm depth in seasons of year one (1) on the kikuyu site, affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1}; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

Kjeldahl N in the deeper depths of 100 – 200 and 200 – 300 mm was similar to what was found in the 0 – 100 mm soil depth. There were no interaction (P>0.05) between treatment and grazing cycle, but grazing cycle did influence (P≤0.05) the Kjeldahl-N in the two deeper depths (Table 3.11). The deeper depths did not, however, follow similar patterns to the 0 – 100 mm in terms of Kjeldahl-N. At the 100 – 200 mm depth, the lowest (P≤0.05) Kjeldahl-N was found during December 2016, with June and July 2016, and January and March 2017 being similar (P>0.05) to the lowest (Figure 3.15). In the 200 – 300 mm depth, Kjeldahl-N was almost equivalent to one another during all grazing cycles, except during August 2016 which was higher (P≤0.05) than the rest (Figure 3.16). The higher Kjeldahl-N in August may be due to two major rainfall events during the previous month (data not shown), which washed the N to deeper depths than to where it would have flowed had there been an even distribution of rain throughout the month.

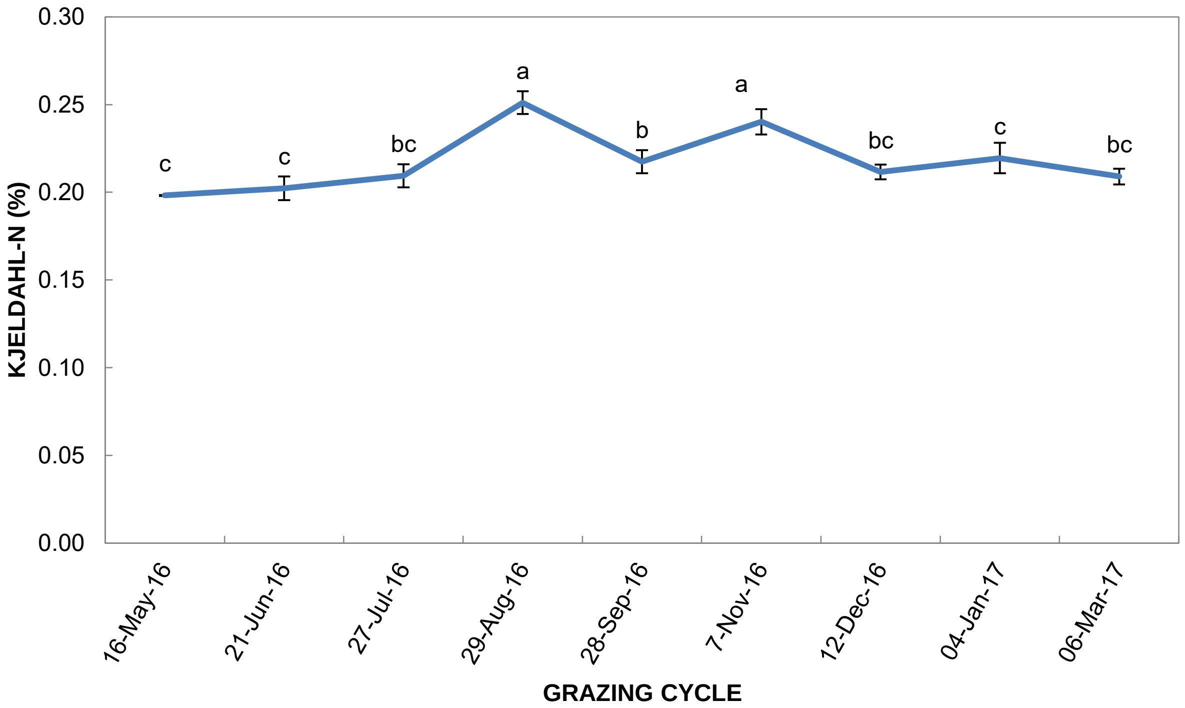

Figure 3.14: Average Kjeldahl soil nitrogen (N) (%) at the 0 - 100 mm depth on the kikuyu site, when averaged over treatments. Error bars indicate standard error. No common letter above data points, indicates significant difference at 5% level

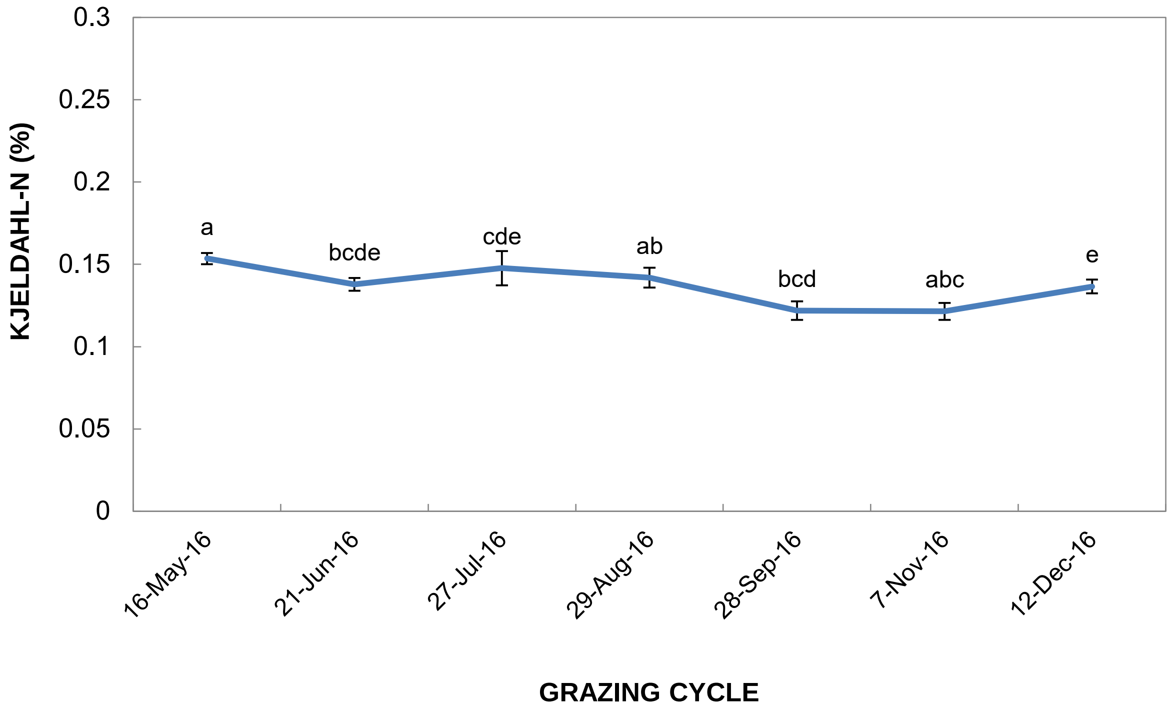

Figure 3.15: Average Kjeldahl soil nitrogen (N) (%) at the 100 - 200 mm depth on the kikuyu site, when averaged over treatments. Error bars indicate standard error. No common letter above data points, indicates significant difference at 5% level

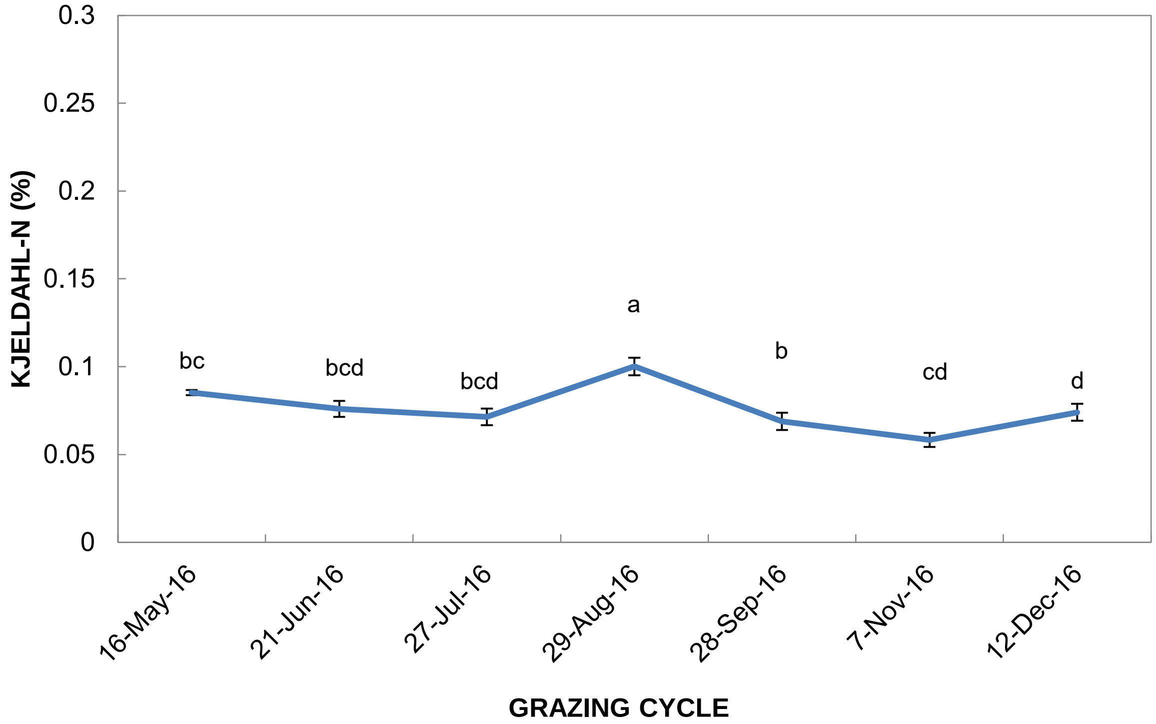

Figure 3.16: Average Kjeldahl soil nitrogen (N) (%) at the 200 – 300 mm depth on the kikuyu site, when averaged over treatments. Error bars indicate standard error. No common letter above data points, indicates significant difference at 5% level

3.3.2 Kikuyu-ryegrass pasture site

3.3.2.1 Total mineral nitrogen, nitrate and potential mineralisable nitrogen

The response of total mineral N in the 0 – 100 mm depth to treatment varied (P≤0.05) in different grazing cycles (Table 3.13). Treatment Nvar (15.1 mg kg^{-1} ± 0.7) and N0 (15.5 mg kg^{-1} ± 0.6) was similar (P>0.05) in soil mineral content, when averaged over grazing cycles (Figure 3.17). Treatments N20 and N40 had similar (P>0.05) total mineral N compared to N0 and Nvar in all grazing cycles, except December 2016. During December 2016, N20 and N40 had a higher (P≤0.05) total mineral N content compared to N0 and Nvar. The reason behind this might be that N accumulated since there was little rainfall during these cycles (Figure 3.1), resulting in N not being leached into deeper soil depths. Another reason might be due to the plants not using the amount of N available in the previous grazing cycle (November 2016 to December 2016). Both treatments N60 and N80 showed an increase in total inorganic N from May to November 2016 (P≤0.05). Treatment N80 then maintained a similar (P>0.05) N content from December to March 2016, after which it decreased (P≤0.05) to a lower value during May and June 2017. Treatment N60 decreased (P≤0.05) from December 2016 to January 2017, but remained similar (P>0.05) until July 2017. During the grazing cycles where the temperature was getting lower and rainfall was high (April to August 2017), compared to the same period in 2016, it is possible to assume that treatment N60 and N80 resulted in nitrate being leached into the soil water. This might be why there were no difference (P>0.05) between the high and low N treatments during May, June, August and to some extent July 2017. During March 2017, the kikuyu-ryegrass site was over-sown with no N application and therefore also slow pasture growth within the establishment period. This might contribute to low mineral N found in May 2017 and also low use of N by young ryegrass seedlings leaving the soil vulnerable to N losses.

From the data relating to total mineral N in the soil, it can be deduced that treatments N60 and N80 were not environmentally sustainable. These high N application rates resulted in high mineral N, which if present in the nitrate form, are able to leach into the deeper soil profile and eventually into the soil water. Although the majority of roots are present in the 0 –100 mm depth, the potential does exist that some of this mineral N would be utilised at deeper soil depths.

Table 3.13: ANOVA of kikuyu-ryegrass site regarding potential mineralisable nitrogen (PMN) in the various depths (Num DF = Numerator degrees of freedom, Den. DF = Denominator degrees of freedom)

	Num. DF	Den. DF	F	P-value
Total Mineral N [1] (0 – 100 mm)				
Treatment	5	18	8.67	<0.001
Grazing cycle	12	212	3.88	<0.001
Treatment* Grazing cycle	60	212	2.30	<0.001
Total Mineral N (100 – 200 mm)				
Treatment	5	18	12.48	<0.001
Grazing cycle	6	108	3.02	0.009
Treatment* Grazing cycle	30	108	1.71	0.024
Total Mineral N (200 – 300 mm)				
Treatment	5	18	16.80	<0.001
Grazing cycle	6	108	3.22	0.006
Treatment* Grazing cycle	30	108	1.57	0.049
PMN [2] (0 – 100 mm)				
Treatment	5	18	0.42	0.832
Grazing cycle	8	144	8.10	<0.001
Treatment* Grazing cycle	40	144	0.76	0.841
PMN (100 – 200 mm)				
Treatment	5	18	0.38	0.855
Grazing cycle	6	108	4.39	0.001
Treatment* Grazing cycle	30	108	0.63	0.928
PMN (200 – 300 mm)				
Treatment	5	18	1.24	0.333
Grazing cycle	6	108	4.99	<0.001
Treatment* Grazing cycle	30	108	0.68	0.886

[1] Total mineral N measured in mg kg^{-1}
() Indicates depth
* Interaction between main effects
[2] PMN measured in kg ha^{-1} grazing $cycle^{-1}$

The response of mineral N varied ($P \leq 0.05$) among treatments and grazing cycles in the 100 – 200 mm depth (Table 3.13). The effects of treatments N0, N20, N40 and Nvar on total mineral N remained similar ($P > 0.05$) from May 2016 to February 2017, with the exception of N40 during September 2016 and N20 during December 2016 (Figure 3.18). Compared with the baseline in

May 2016, treatment N60 and N80 again displayed increased ($P \leq 0.05$) total mineral N content as the study continued. This is a sign of a build-up of mineral N not being utilised by the pasture plants, even in the deeper soil layers.

Total mineral N in the 200 – 300 mm depth varied ($P \leq 0.05$) with treatments according to grazing cycle (Table 3.13). Treatment N60 and N80 had a higher ($P \leq 0.05$) total mineral N during September, November, December 2016 and February 2017 compared to May 2016. Treatments N0, N20, N40 and Nvar remained similar ($P > 0.05$) throughout the grazing cycle compared to the total mineral N content observed during May, indicating that at these rates mineral N was not accumulating in the deeper layers and that leaching was likely limited. In this depth, it was N60 which had the highest ($P \leq 0.05$) mineral N and not N80 as in the shallower depths. One might argue that the higher N application rate allows for higher possibility of leaching, and that leaching already occurred in the N80 treatment. However, irrespective of this trend, based on the relatively high mineral N content in the deeper soil layers (where N is relatively unavailable to shallow rooted pasture species) of the N60 and N80 treatments, these high rates of fertilisation would not be recommended.

As discussed in a previous section (Kikuyu, 3.3.1.1), the WFD did not work as well as in a study conducted by Fessehazion et al. (2011) on Cedara. In the current study, even with no applied N, the nitrate concentration of the 300 mm depth WFD was higher than the recommended allowable nitrate concentration of 50 mg L^{-1} (EEC 1991) in soil water or 5 mg L^{-1} nitrate-N (DWAF 1996) in drinking water (Figure 3.20). These results could be indicative that the WFD and current guidelines for its use may not be as effective on all pasture systems or varying soil type, highlighting the need for site specific N fertilisation studies in order to prevent the pollution of underground water sources. As seen in Figure 3.19, it was N0 and Nvar which consistently had the lowest total mineral N content in the 200 – 300 mm depth throughout the study. Treatment N20 was similar ($P > 0.05$) during most grazing cycles. Treatment N40 ($P > 0.05$) had similarly low mineral N, but not during August and September 2016 ($P \leq 0.05$). The WFD results suggest that the high nitrate concentration in the 150 mm depth (>250 mg nitrate L^{-1}) and in the 300 mm depth (>130 mg nitrate L^{-1}) suggest that N was not fully utilised, in this system, even at low rates of N fertilisation.

The total mineral N in the soil comprises of nitrate and ammonium. Ammonium may be converted to ammonia, which could be lost through volatilisation. Both urine patches and N fertiliser application increase the possibility of volatilisation losses (McKenzie and Tainton 1993; Thompson and Fillery 1998). Animal excreta on pasture results in volatilisation losses of between 3 to 8%, but this amount may increases by up to five times when fertiliser is applied at a rate of 400 kg N ha^{-1} $year^{-1}$ (Ledgard et al. 1999). Based on the above research findings and the results of the current study, it is concluded that treatment N60 and N80 would likely result in an inefficient N use, financial loss and a detrimental losses of N into the environment.

Grazing cycle as a main effect influenced (P≤0.05) (Table 3.13) the PMN at all the depths. However, treatment did not affect the PMN (P>0.05). At the 0 – 100 mm depth, it was September 2016 and May 2017 which had the highest mineralisation rates (P≤0.05) (Figure 3.21). There was no clear pattern of mineralisation over years or on a seasonal basis.

There was no PMN response in terms treatment (P>0.05) at the 100 – 200 mm and 200 – 300 mm depths within grazing cycles (Table 3.13

). The grazing cycle was the main factor that determined (P≤0.05) the mineralisation of N in both depths. In the 100 – 200 mm depth, it was found that during September, November and December 2016 the highest rates occurred (Figure 3.22). Although the PMN was higher (P≤0.05) during November and December than during other months at the 200 – 300 mm, it was lower (P≤0.05) during September. November and December were similar (P≤0.05) to August in the 200 – 300 mm. Thus the trend was similar, but not exactly the same as at 100 – 200 mm (Figure 3.23). According to Fulkerson et al. (2011), mineralisation in temperate climates can account for 20 – 120 kg N ha^{-1} year^{-1} (Fulkerson et al. 2011). This is similar to the range found in the current study.

The lack of response in terms of total inorganic N and PMN at the extremes of N fertilisation regimes applied during this study is unexpected. Stout et al. (1997) reported that N reserves in the soil were depleted by growing a barley-annual ryegrass intercrop without N fertiliser application. This particular study of Stout et al. (1997) was undertaken to evaluate the response to N fertiliser in combination with legumes. On the current study site, the soil was previously managed as it would be on commercial farms in the region. Depleting the soil of N reserves prior to the onset of N application treatments might give a better indication of the effect of fertiliser on the particular pasture. However it would not have been a true representation of the current status of pasture soils in the southern Cape area, since most of them have applied N to their pasture for a number of years. The characterisation of the impact of accumulated N in the systems needs to be improved under these no-till pasture systems. However, results did indicate that N fertilisation could potentially be managed according to seasonal herbage requirements, potentially linked to requirements based on varying growth rates.

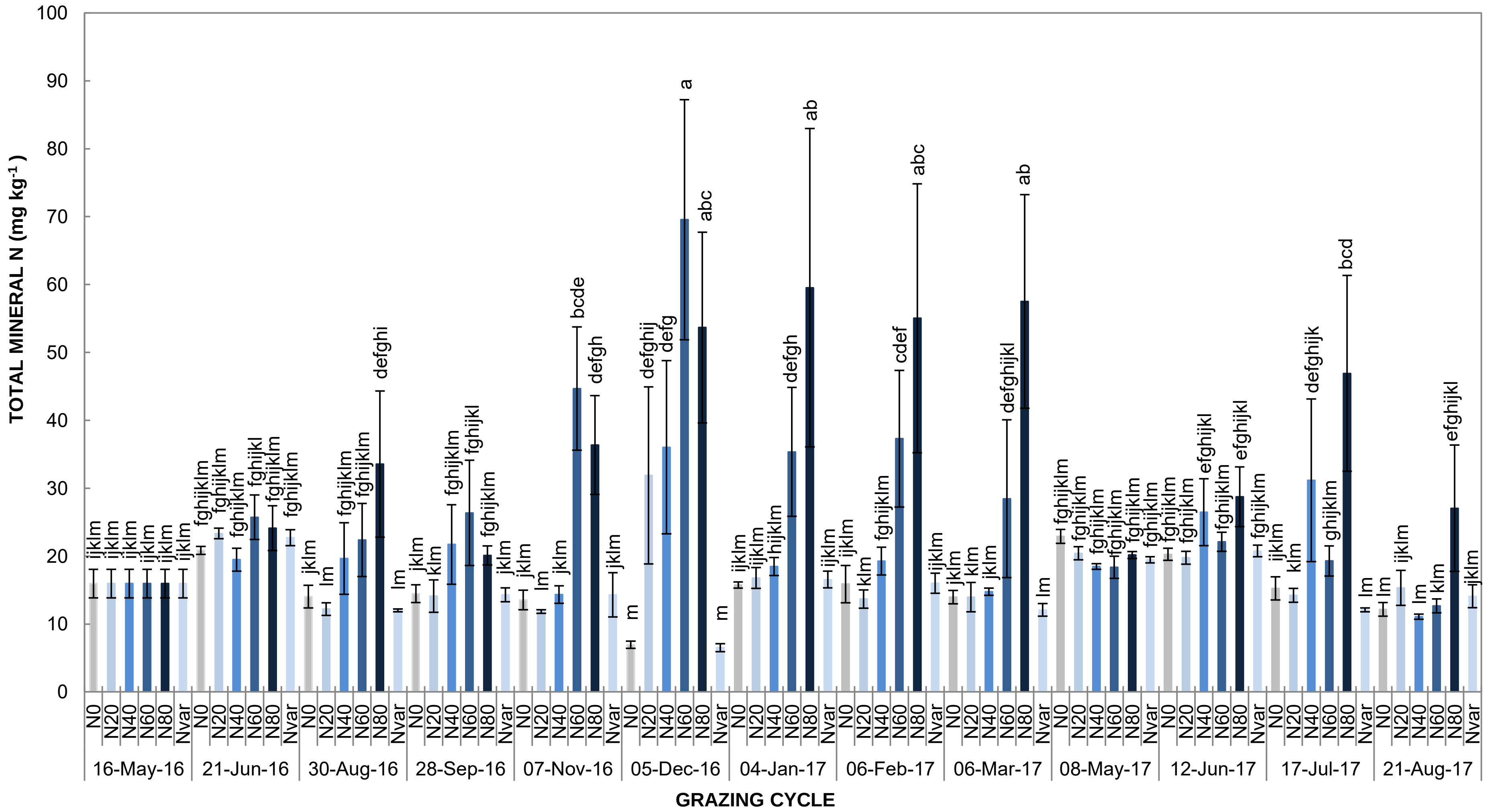

Figure 3.17: Total mineral N (mg kg^{-1}) on the day of sampling at the 0 - 100 mm soil depth on the kikuyu-ryegrass site, as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1}; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

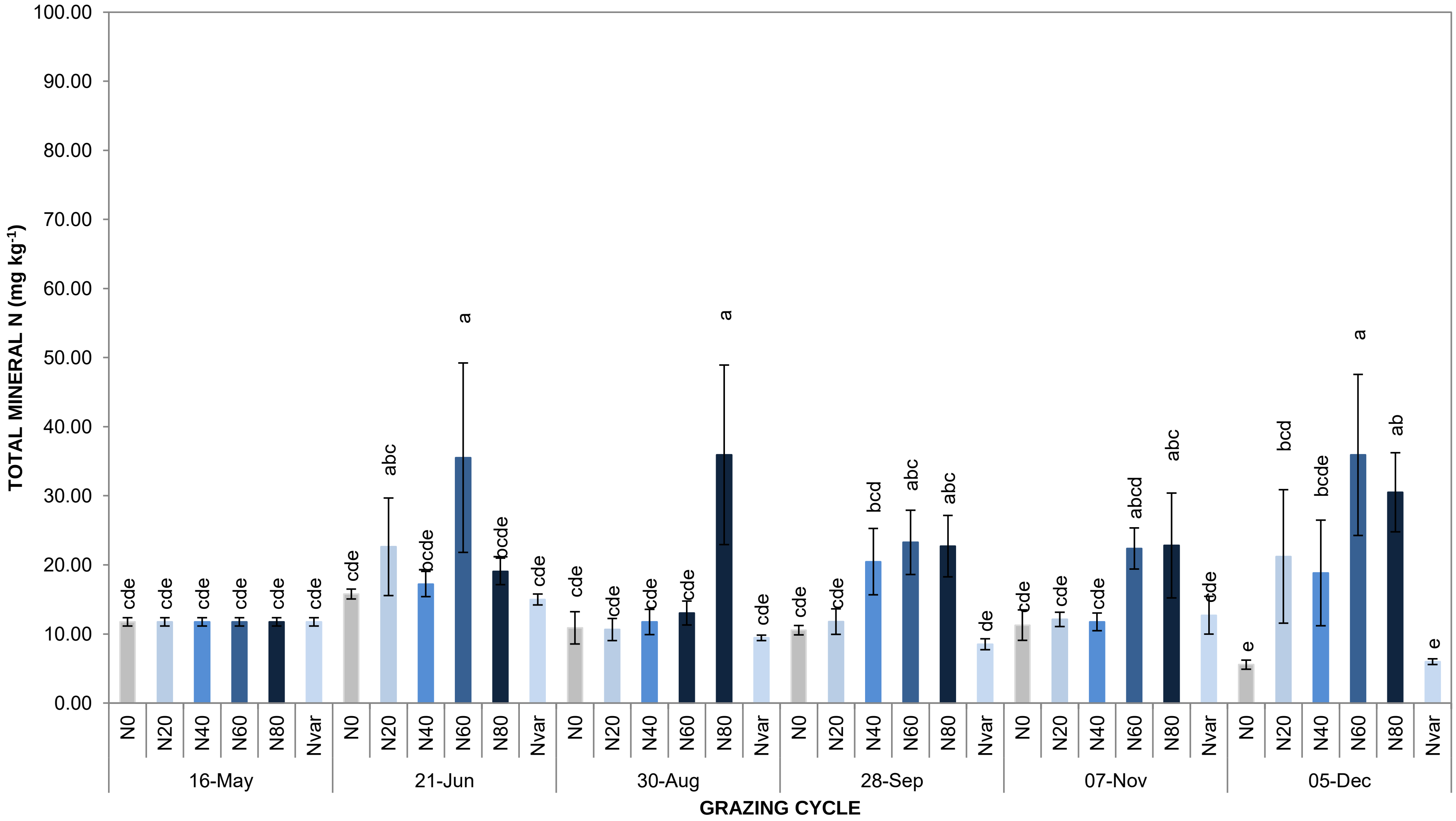

Figure 3.18: Total mineral N (mg kg^{-1}) on day of sampling at the 100 – 200 mm depth, in the kikuyu-ryegrass site, affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

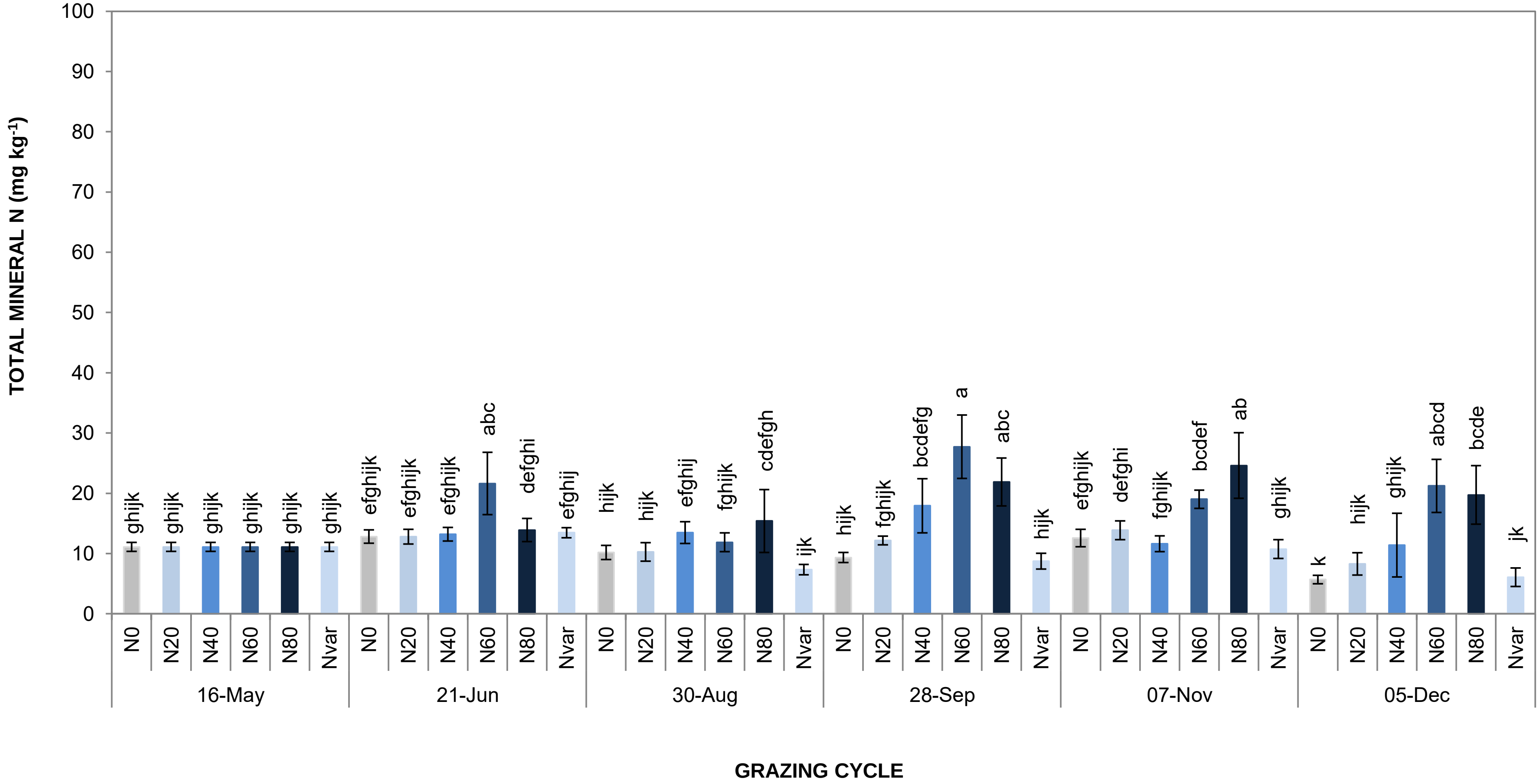

Figure 3.19: Total mineral soil N (mg kg^{-1}) on day of sampling at soil depth 200 - 300 mm on the kikuyu-ryegrass site. N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1}; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

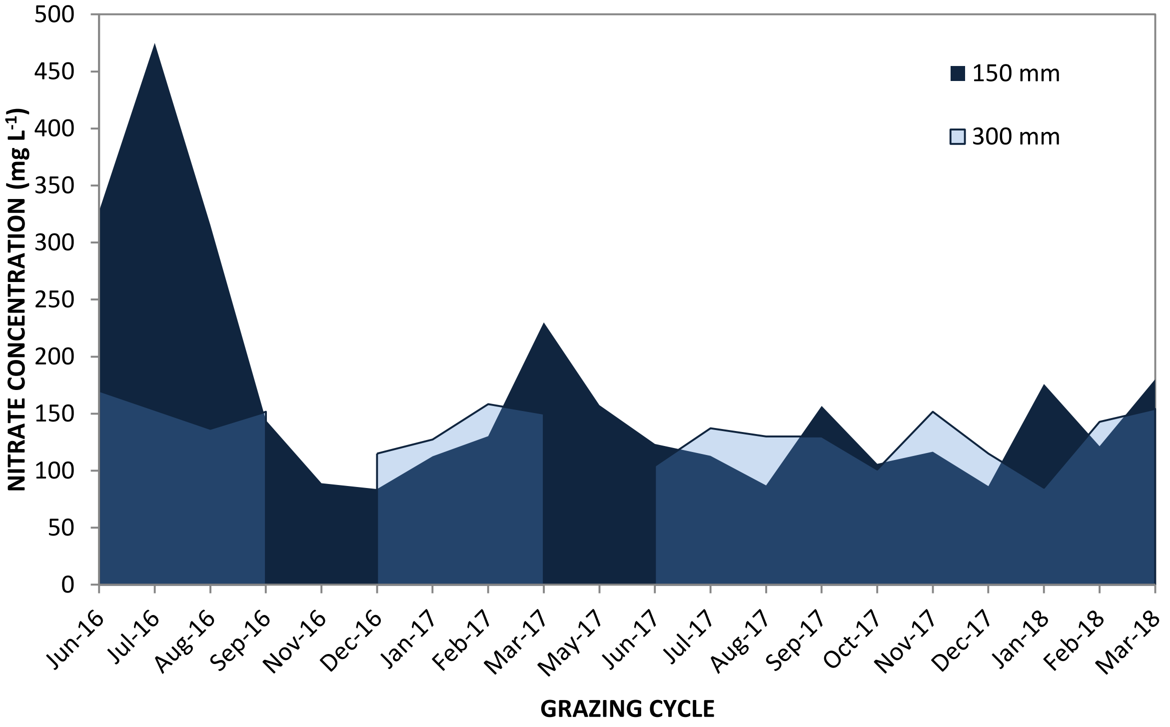

Figure 3.20: Average nitrate concentration (mg nitrate L^{-1}) in soil water collected from wetting front detectors (WFD) for the duration of the trial on the kikuyu-ryegrass site. WFD were used in Nvar plots only, at and installed at 150 mm and 300 mm depth in the soil

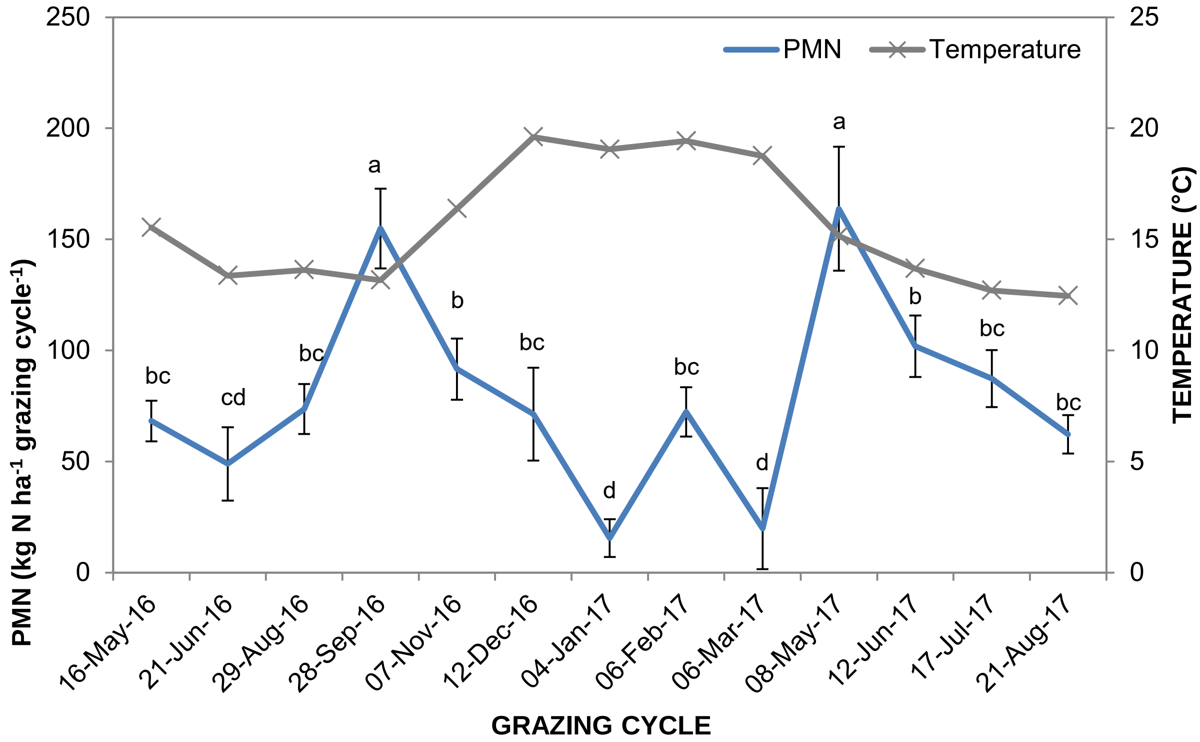

Figure 3.21: Average potential mineralisable nitrogen (PMN) (kg N ha^{-1} grazing $cycle^{-1}$), averaged over all treatments, at the 0 – 100 mm depth on the kikuyu-ryegrass site as affected by grazing cycle. Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level. The average monthly temperatures (°C) are also displayed

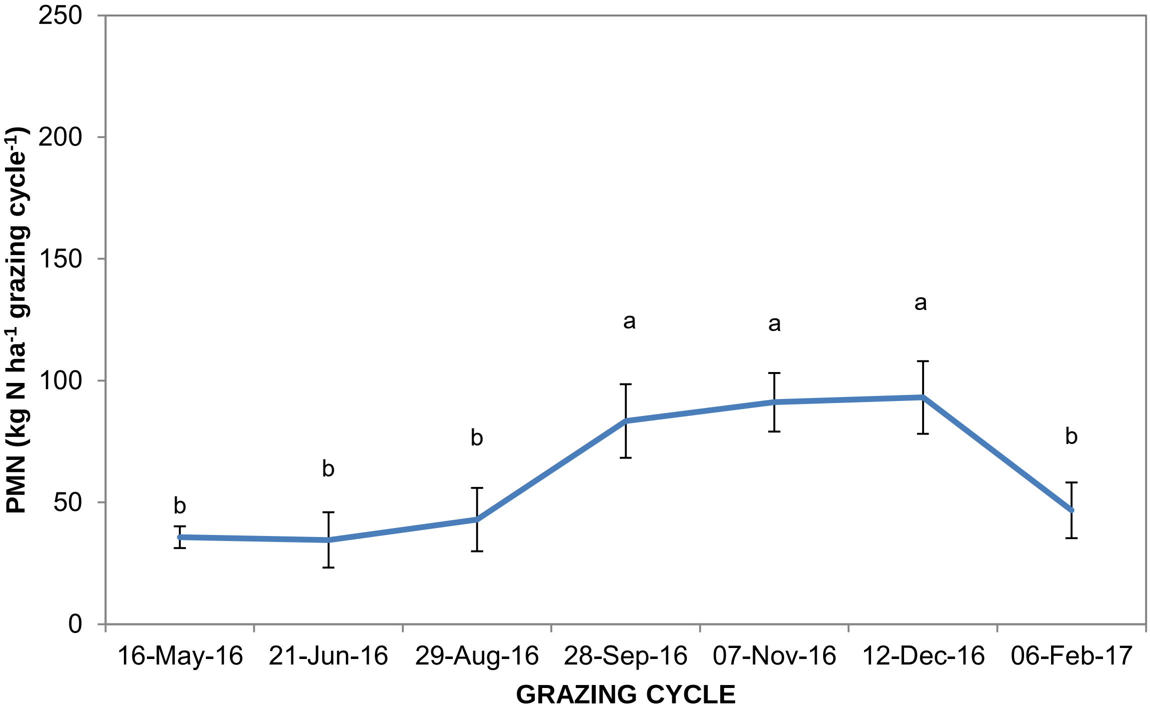

Figure 3.22: Average potential mineralisable nitrogen (PMN) (kg N ha^{-1} grazing cycle^{-1}), averaged over all treatments, at the 100 – 200 mm depth on the kikuyu-ryegrass site as affected by grazing cycle. Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level

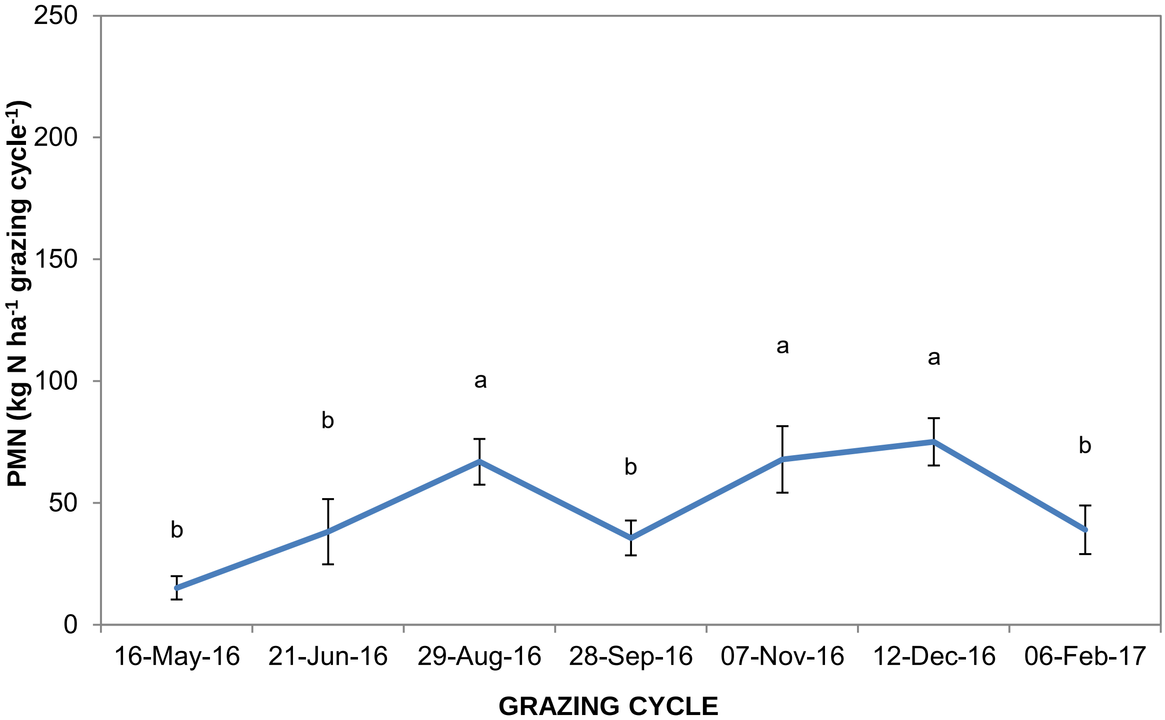

Figure 3.23: Average potential mineralisable nitrogen (PMN) (kg N ha^{-1} grazing cycle^{-1}), averaged over all treatments, at the 200 – 300 mm depth on the kikuyu-ryegrass site as affected by grazing cycle. Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level

3.3.2.2 Carbon to nitrogen ratio

There were no interaction (P>0.05) between treatments and grazing cycles (Table 3.14). When treatments were averaged and compared according to the grazing cycle (Figure 3.24) it showed the highest (P≤0.05) C:N ratio occurred during June 2016 followed by May 2016. After June 2016 the ratio decreased (P≤0.05) from 23.5 to a value of 10.9 in July 2016. The C:N ratio then varied between 10.9 to 16.0 until January 2017.

Table 3.14: ANOVA table of kikuyu-ryegrass site regarding carbon:nitrogen (C:N) ratio in the various depths (Num DF = Numerator degrees of freedom, Den. DF = Denominator degrees of freedom)

	Num. DF	Den. DF	F	P-value
C:N ratio (0 – 100 mm)				
Treatment	5	18	0.57	0.721
Grazing cycle	6	106	116.09	<0.001
Treatment* Grazing cycle	30	106	0.62	0.935
C:N (100 – 200 mm)				
Treatment	5	18	0.21	0.955
Grazing cycle	2	36	20.93	<0.001
Treatment* Grazing cycle	10	36	0.58	0.822
C:N (200 – 300 mm)				
Treatment	5	18	0.80	0.562
Grazing cycle	2	36	5.01	0.012
Treatment* Grazing cycle	10	36	0.32	0.971

() Indicates depth

* Interaction between main effects

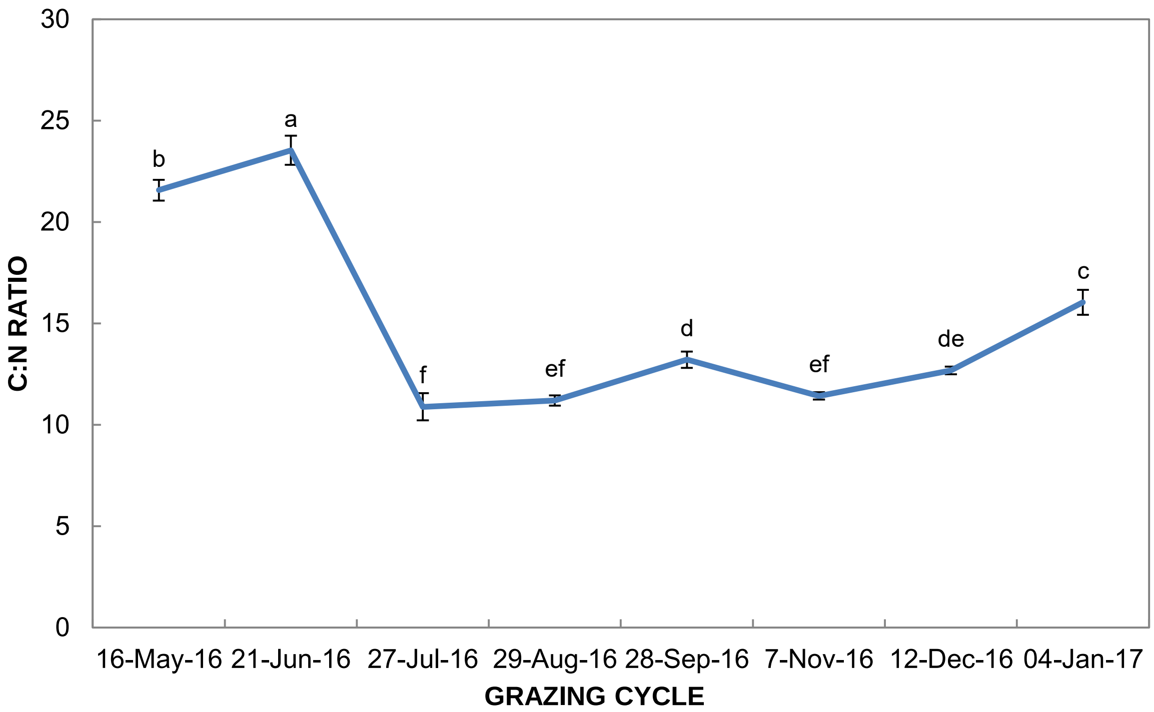

Figure 3.24: Average carbon to nitrogen (C:N) ratio at the 0 – 100 mm depth on the kikuyu-ryegrass site as affected by grazing cycle and averaged over all treatments. Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level

It was found that in the southern Cape, the C:N ratio of cultivated pastures was 11.1 ± 0.3, 11.2 ± 0.3 and 12.1 ± 0.7 in the respective depths of 0 – 100 mm, 100 – 200 mm and 200 – 300 mm (Swanepoel et al. 2014b). This is lower than that of the current study which was 15.1 ± 0.4, 15.4 ± 0.7 and 14.6 ± 0.6 when averaged over the study period for the respective depths of 0 – 100 mm, 100 – 200 mm and 200 – 300 mm.

3.3.2.3 Urease activity

The response of UA to treatments were similar ($P>0.05$) in all grazing cycles (Table 3.15). There were no treatment effects ($P>0.05$) between July and September 2016 (Figure 3.25). Treatment N20 showed an increase ($P\leq0.05$) from September to December, while treatment N80 showed a decrease ($P\leq0.05$) during the same period. All other treatments remained similar ($P>0.05$) from September to December 2016. Most of the variation within UA occurred during 2016. During December 2016 N20 had a higher ($P\leq0.05$) UA than N0, N60 and N80, but similar to N40 and Nvar. This was not however carried through into February 2017, when Nvar had a higher UA than N20, but similar ($P>0.05$) to the rest. Treatments within July 2016, September 2016, May 2017 and July 2017 were similar ($P>0.05$). In the same location, during October 2011, UA was found to be 538 µg NH_4-N g^{-1} $2h^{-1}$ in the 0 - 100 mm depth (Swanepoel et al. 2014b), higher than that of the current study which had a mean UA of 284 µg NH_4-N g^{-1} 2 h^{-1} when averaged for the study period and treatments.

Table 3.15: ANOVA of kikuyu-ryegrass site regarding urease activity in the 0 - 100 mm depth (Num DF = Numerator degrees of freedom, Den. DF = Denominator degrees of freedom)

	Num. DF	Den. DF	F	P-value
Urease activity[1]				
Treatment	5	18	0.28	0.920
Grazing cycle	5	90	3.90	0.003
Treatment* Grazing cycle	25	90	1.53	0.076
Urease activity (normalised)[2]				
Treatment	4	68	6.11	<0.001
Grazing cycle	5	18	10.67	<0.001
Treatment* Grazing cycle	20	68	5.51	<0.001

[1] measured in µg NH_4-N g^{-1} $2h^{-1}$

[2] measured in µg NH_4-N g^{-1} N $2h^{-1}$

() Interaction between main effect

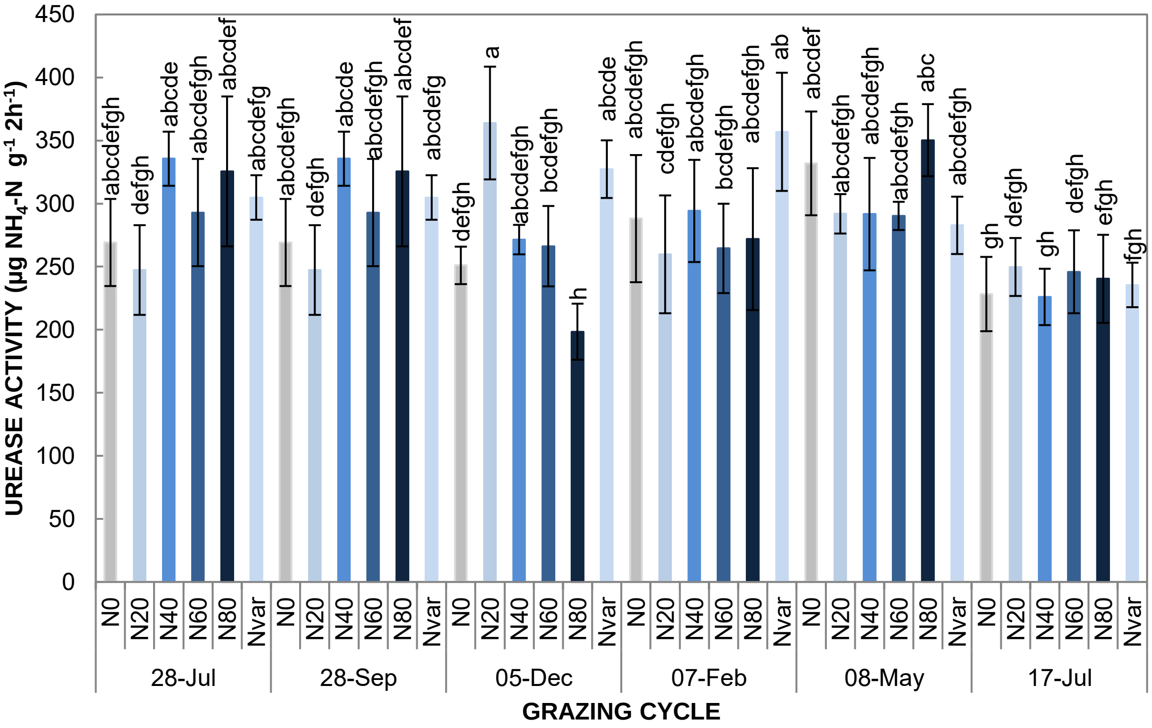

Figure 3.25: Urease activity (µg NH_4-N g^{-1} $2h^{-1}$) at the 0 - 100 mm soil depth on the kikuyu-ryegrass site approximately every second grazing cycle affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

When the UA data was normalised with mineral N, the results were considerably different. The response of normalised UA to treatments varied (P≤0.05) between grazing cycle in the 0 – 100 mm depth (Table 3.15). According to these results during the warmer grazing cycles (December 2016 and February 2017), the low N treatments (N0, N20, Nvar) had a greater (P≤0.05) activity compared to the high N treatments (N60 and N80). During the cooler grazing cycles, September 2016 and May 2017, there were no differences (P>0.05) between treatments. Cartes et al. (2009) found differences in UA amongst the two soils they studied. The andisol soil showed an increase in UA as the temperature was increased (Cartes et al. 2009). This is similar to what was seen in the normalised UA values during December 2016 especially, and to a lesser extent February 2017, during which the temperatures was highest (20°C) or similar to the highest (19°C). Similar to the discussion in the previous section (3.3.1.3) regarding the redundant production of the urease in chemically fertilised pastures, this can also be applicable on this kikuyu-ryegrass study site.

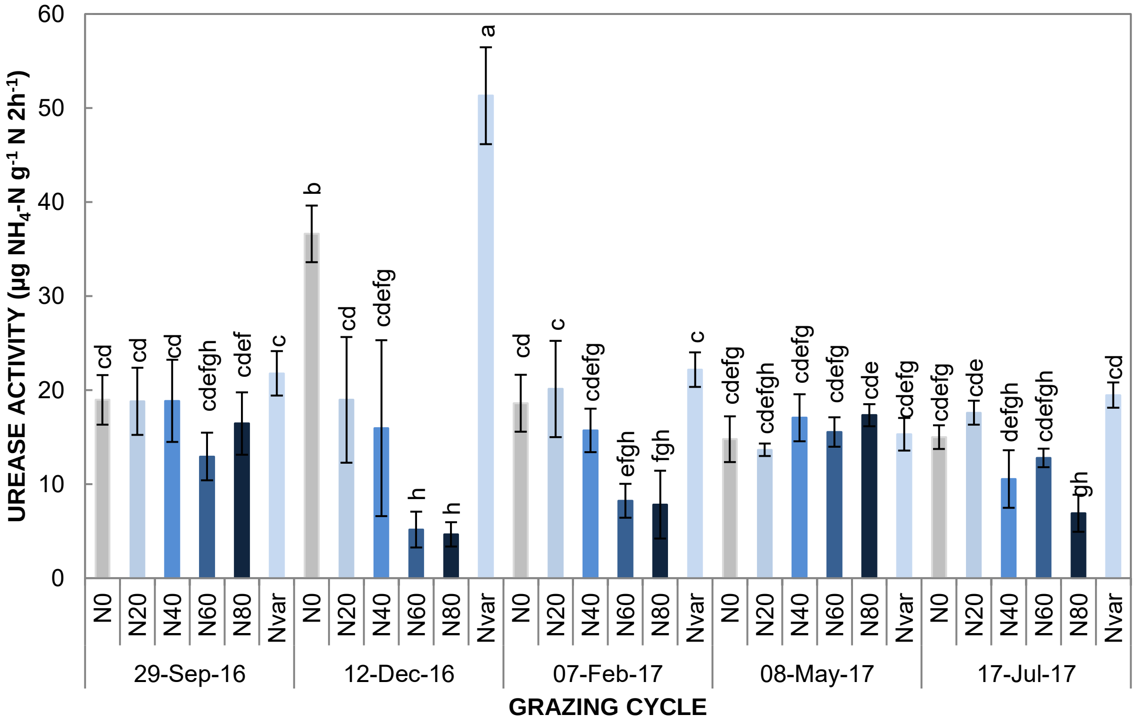

Figure 3.26: Normalised Urease Activity (µg NH_4-N g^{-1} N $2h^{-1}$) in the 0 - 100 mm soil depth on the kikuyu-ryegrass site approximately every second grazing cycle as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

3.3.2.4 Leco-N and Kjeldahl-N

Both Leco-N (0 – 100 mm depth) and Kjeldahl-N (all depths) were influenced (P≤0.05) by grazing cycle rather than treatments (P>0.05). These results are shown in Table 3.16 and

Table 3.17 and also Figure 3.27, Figure 3.28, Figure 3.29 and Figure 3.30.

From July until November 2016 the highest (P≤0.05) or similar (P>0.05) to the highest Kjeldahl-N content were found compared to baseline (May 2016) Kjeldahl-N in the 0 – 100 mm depth (Figure 3.28). The baseline values are those obtained prior to application of N treatment during May 2016. In the 100 – 200 mm depth, the lowest or similar to the lowest Kjeldahl N was found during September and November 2016, while May, July and August 2016 having the highest or similar to the highest amount (Figure 3.29). Kjeldahl-N in the 200 – 300 mm depth was also lowest (P≤0.05) in November 2016 and highest (P≤0.05) in August 2016. It is noted that an inverse effect is found between Kjeldahl values of 0 – 100 mm and 200 – 300 mm depths (Figure 3.30).

Total N (Kjeldahl-N) was previously measured at 0.18% at the same location as the current study (Swanepoel et al. 2015b). Hadas et al. (2004) also found kjeldahl values of 0.14%, in a soil under long term irrigated crops amended cattle manure. Results of the current study were 0.22%, 0.14% and 0.08% for the depths of 0 – 100 mm, 100 – 200 mm and 200 – 300 mm depths respectively.

Table 3.16: ANOVA table of kikuyu-ryegrass site regarding Leco-Nitrogen (N) in the various depths (Num DF = Numerator degrees of freedom, Den. DF = Denominator degrees of freedom)

	Num. DF	Den. DF	F	P-value
Leco-N (0 – 100 mm)				
Treatment	5	18	0.48	0.785
Grazing cycle	11	197	134.44	<0.001
Treatment*grazing cycle	55	197	1.11	0.297
Leco-N (0 – 100 mm)				
Treatment	5	18	0.72	0.614
Season	3	53	146.88	<0.001
Treatment*Season	15	53	1.32	0.223
Leco-N (100 – 200 mm)				
Treatment	5	18	0.35	0.878
Season	3	54	40.66	<0.001
Treatment*Season	15	54	0.89	0.574
Leco-N (200 – 300 mm)				
Treatment	5	18	0.51	0.763
Season	3	53	29.17	<0.001
Treatment*Season	15	53	0.67	0.803

[1] measured as %
() indicates depth
*Interaction between main effects

Table 3.17: ANOVA table of kikuyu-ryegrass site regarding Kjeldahl-Nitrogen (N) in the various depths (Num DF = Numerator degrees of freedom, Den. DF = Denominator degrees of freedom)

	Num. DF	Den. DF	F	P
Kjeldahl-N (0 – 100 mm)				
Treatment	5	18	0.62	0.683
Grazing cycle	6	108	10.22	<0.001
Treatment*grazing cycle	30	108	1.17	0.278
Kjeldahl-N (100 – 200 mm)				
Treatment	5	18	1.14	0.378
Grazing cycle	6	108	5.31	<0.001
Treatment*grazing cycle	30	108	0.75	0.810
Kjeldahl-N (200 – 300 mm)				
Treatment	5	18	0.34	0.881
Grazing cycle	6	108	15.67	<0.001
Treatment*grazing cycle	30	108	0.91	0.605

[1] measured as %
() indicates depth
*Interaction between main effects

The response of Leco-N to treatments were similar (P>0.05) in the respective seasons in all depths, 0 – 100 mm, 100 – 200 mm and 200 – 300 mm (Table 3.16). The differences in Leco-N were due to season (P≤0.05). When soil N was averaged over treatments, it showed clear seasonal effects, decreasing steadily from winter to autumn, and then increasing again from autumn to winter (Figure 3.27). In the 0 – 100 mm depth, treatments did not differ (P>0.05) in regard to Leco-N for winter and spring of the first year, with the exception of N60 and Nvar for which Leco-N (total N) decreased (P≤0.05) from winter to spring (Figure 3.31). All treatments then decreased (P≤0.05) from spring to summer and were similar (P>0.05) during summer and autumn. The exception was that N40 and N80 decreased (P≤0.05) from summer and autumn. The observed decline in soil N from spring to summer coincided with high pasture accumulation rates, and was thus likely associated with an increase in the N requirement of the pasture.

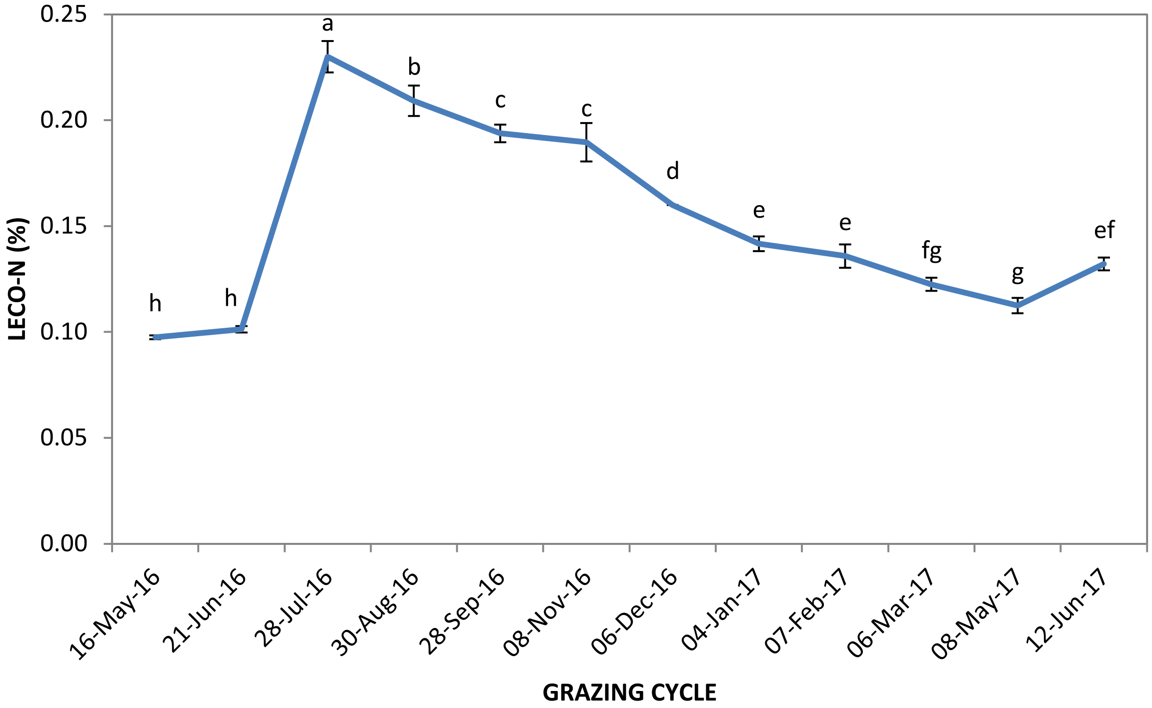

Figure 3.27: Average Leco nitrogen (N) (%) at the 0 – 100 mm depth on the kikuyu-ryegrass site, when averaged over all treatments. Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level

In terms of Leco-N at the 100 – 200 mm depth, winter was similar (P>0.05) to spring, with the exception of N60 and N40 which decreased (P≤0.05) (Figure 3.32). The spring total soil N of N40 and N60 remained similar (P>0.05) during spring and summer, while it increased (P≤0.05) from spring to summer for the remaining treatments. All the treatments remained similar (P>0.05) from summer to autumn.

The Leco-N in the 200 – 300 mm depth was similar (P>0.05) during winter and spring, except N60 and Nvar which decreased (P≤0.05) within this period (Figure 3.33). During spring the total soil N content of treatments was higher (P≤0.05) than during summer, with the exception of treatment N20, for which it remained similar (P>0.05). Treatment effects on Leco-N at the 200 – 300 mm depth were similar (P>0.05) within summer and autumn and also similar (P>0.05) within spring and autumn.

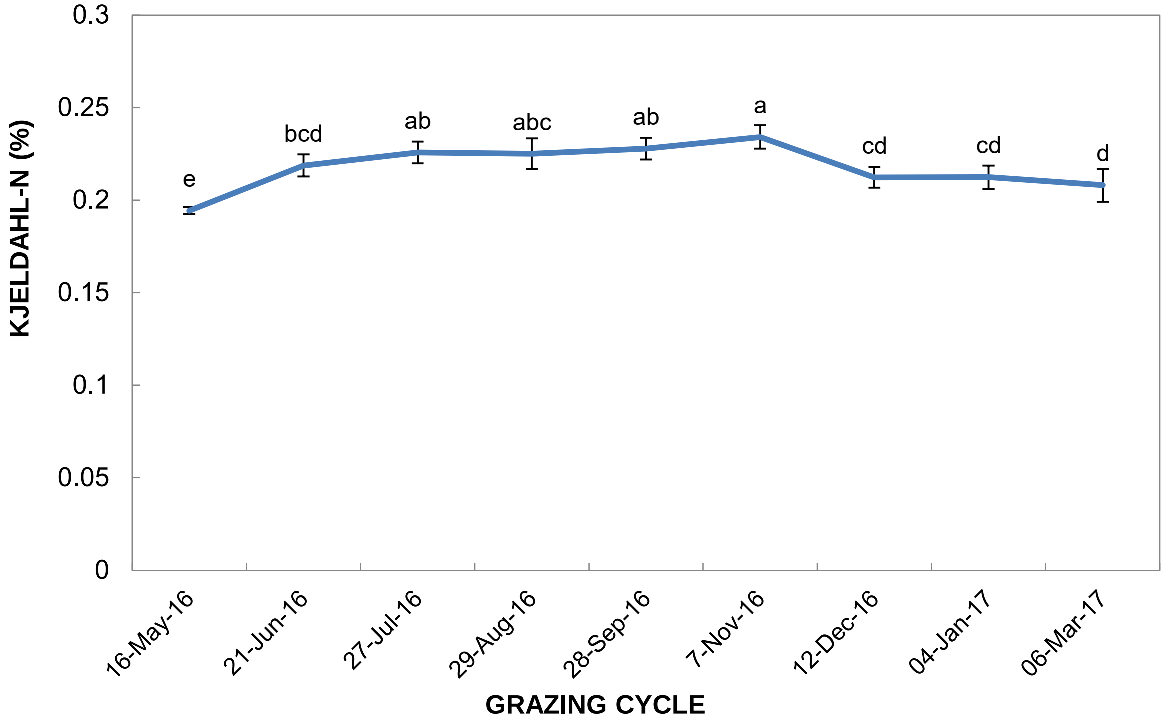

Figure 3.28: Average Kjeldahl soil nitrogen (N) (%) at the 0 - 100 mm depth on the kikuyu-ryegrass site, when averaged over treatments. Error bars indicate standard error. No common letter above data points, indicates significant difference at 5% level

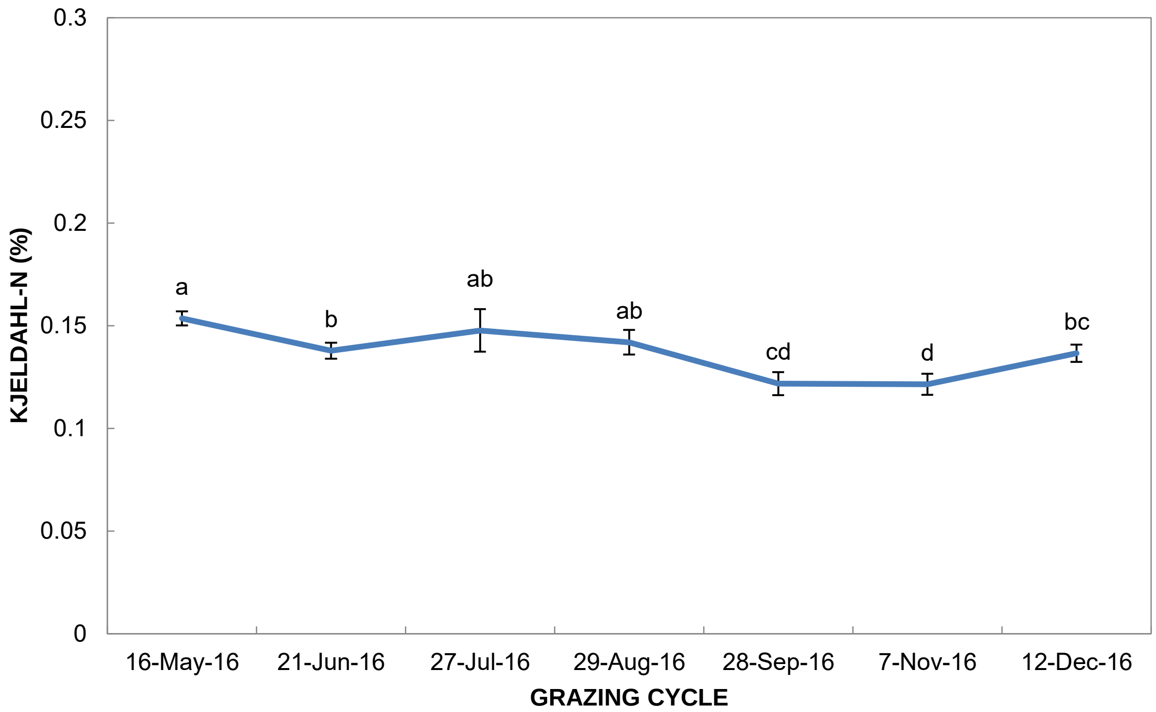

Figure 3.29: Average Kjeldahl soil nitrogen (N) (%) at the 100 – 200 mm depth on the kikuyu-ryegrass site, when averaged over treatments. Error bars indicate standard error. No common letter above data points, indicates significant difference at 5% level

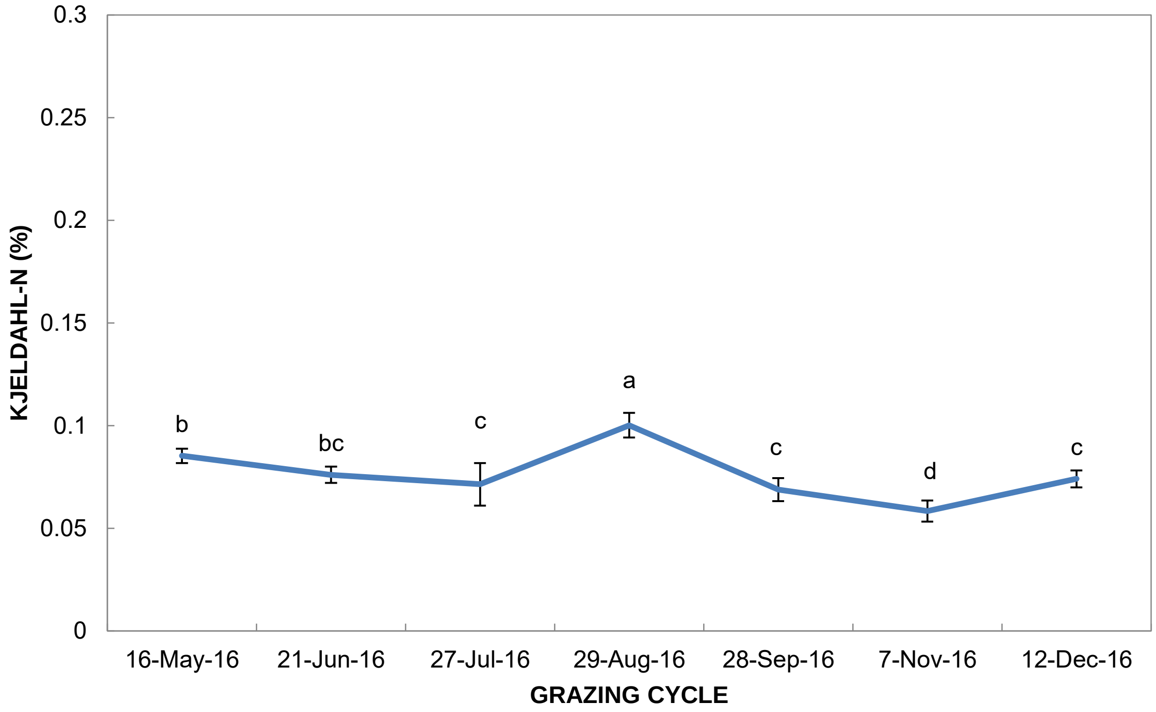

Figure 3.30: Average Kjeldahl soil nitrogen (N) (%) at the 200 – 300 mm depth on the kikuyu-ryegrass site, when averaged over treatments. Error bars indicate standard error. No common letter above data points, indicates significant difference at 5% level

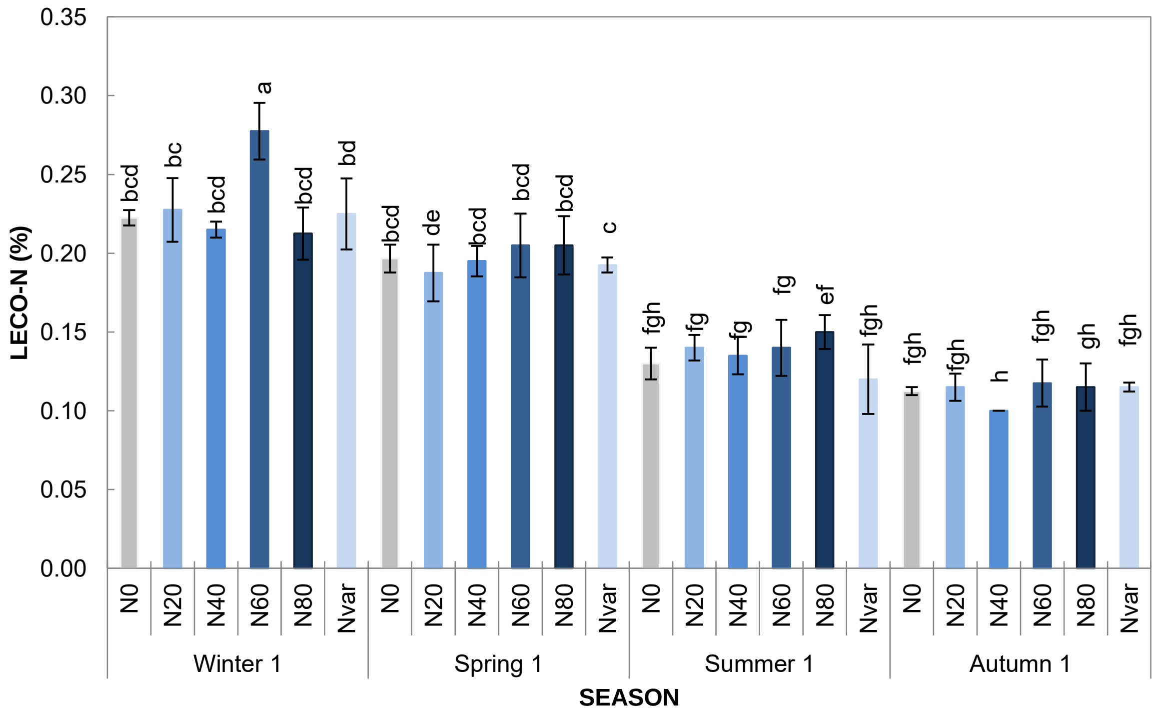

Figure 3.31: Leco nitrogen (N) (%) at the 0 – 100 mm depth in seasons of year one (1) on the kikuyu-ryegrass site, affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

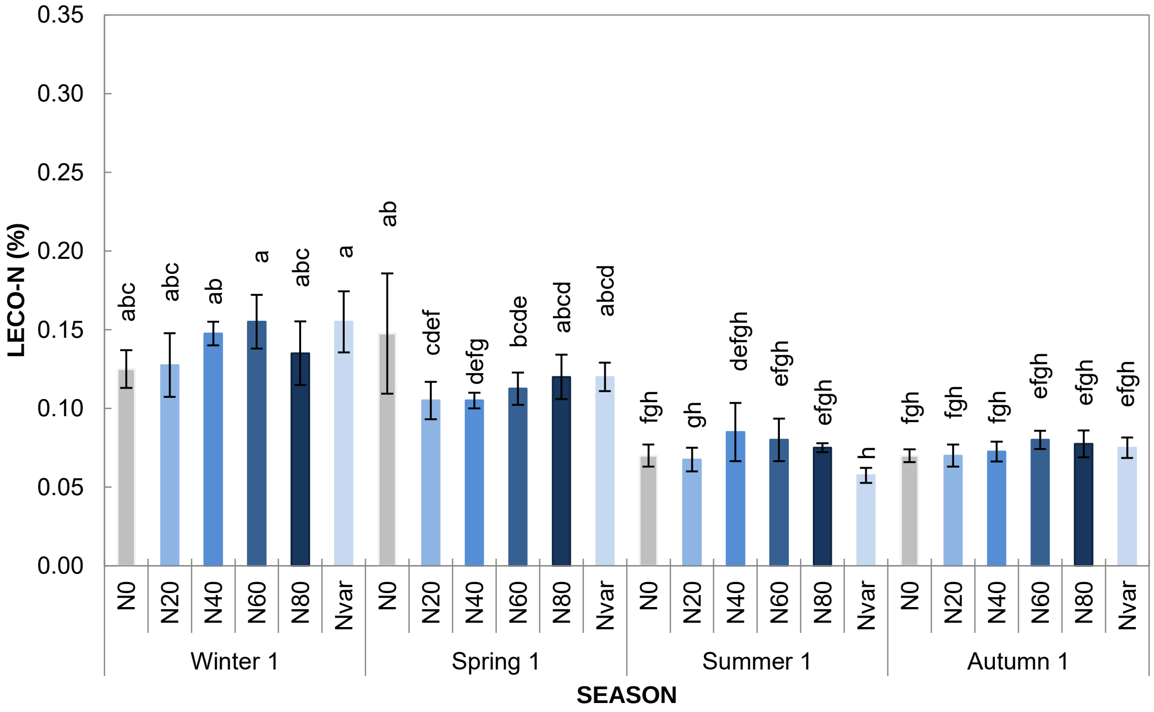

Figure 3.32: Leco nitrogen (N) (%) at the 100 – 200 mm depth in seasons of year one (1) on the kikuyu-ryegrass site, affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

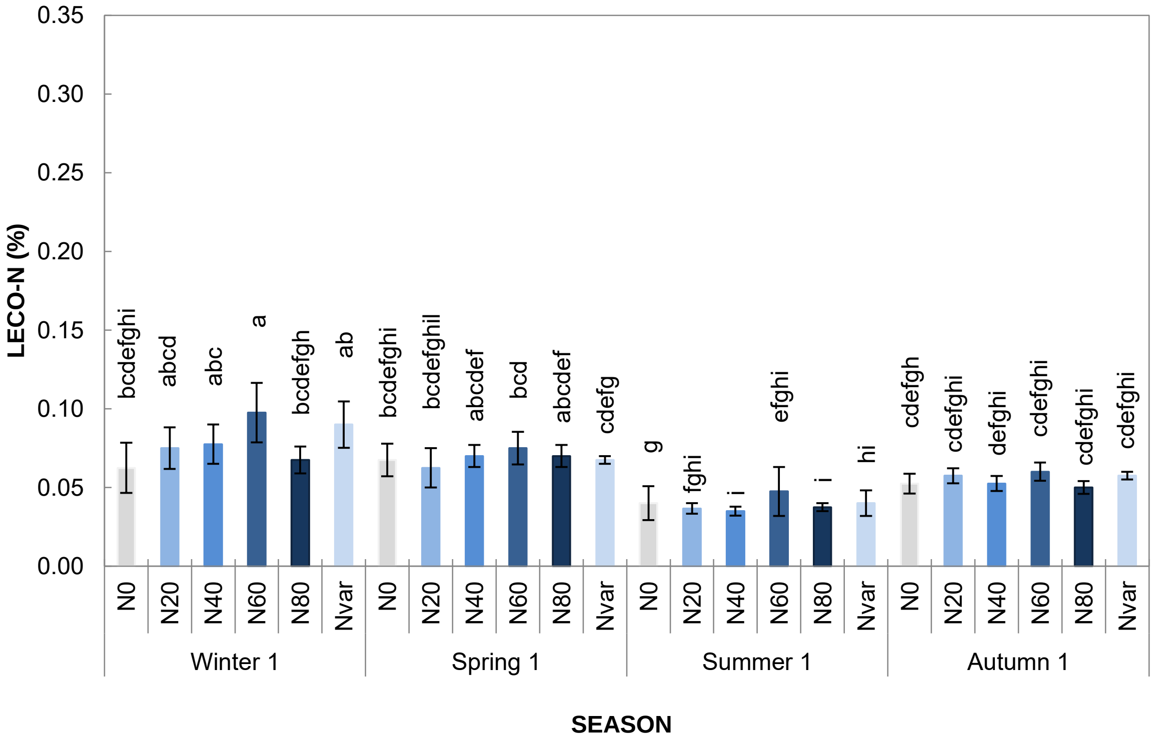

Figure 3.33: Leco nitrogen (N) (%) at the 200 – 300 mm depth in seasons of year one (1) on the kikuyu-ryegrass site, affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$; Nvar = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars, indicates significant difference at 5% level

Leco-N determined in a New Zealand non-irrigated pasture, grazed by sheep and consisting of a mixture of pasture species, including perennial ryegrass (*Lolium perenne*), was much higher than the results reported during the current study. The range of Leco-N was 0.41 to 0.44% as yearly average (Ross et al. 2004) compared to the current studies which ranged between 0.10 to 0.28%. In Serbia, Leco-N of a Italian ryegrass pasture in the 0 – 300 mm depth was 0.197% (Simić et al. 2009). In a 12 year trial regarding tillage, N fertiliser and cropping system (various spring wheat, sunflower rotations), N treatment did not affect Leco-N in the 0 – 76 mm depth (Halvorson et al. 2002). The aforementioned result were comparable to the current study, were treatment differences within seasons were minimal. The exception was during winter of year one which had a higher ($P \leq 0.05$) Leco-N compared to the other treatments.

3.4 Conclusion

Total mineral N content in soil responded to N treatment. Applying >40 kg N ha^{-1} resulted in a build-up of mineral N in the soil profile (up to 300 mm) to a point where it surpass the requirement of the pasture. Because plants cannot utilise all the N in treatments >40 kg N ha^{-1}, N is vulnerable to leaching and volatilisation losses, which pose an environmental risk and has an economic implication. High rates of N fertilisation (>40 kg N ha^{-1}) also affected microbial activity as urease activity were lower when fertilisation were high. Urease enzyme activity became redundant when mineral N was high. Redundancy of urease activity, and therefore a reduced microbial activity, is more evident at N rates of >40 kg N ha^{-1} and is not recommended.

Even though treatment did not have an effect on the potential of the soil to mineralise N, the soil had a large potential to mineralise N, which could at least partially replace N from fertilisation. On the kikuyu site PMN was in the range of 50 – 170 kg ha^{-1} grazing cycle^{-1}, while on the kikuyu ryegrass site this range was 15 – 150 kg ha^{-1} grazing cycle^{-1}. The soil thus has the ability to supply the plants with a significant amount of N. A fertilisation programme that takes the potential of the soil to supply N into account, should be developed.

3.5 References

Addiscott TM. 1996. Fertilizers and Nitrate Leaching. *Issues in Environmental Science Technology* 5: 1–26.

Adetunji AT, Lewu FB, Mulidzi R, Ncube B, Studies O, Cape W. 2017. The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: A review. *Journal of Soil Science and Plant Nutrition* 17: 794–807.

Baligar VC, Fageria NK, He ZL. 2001. Nutrient Use Efficiency in Plants. *Communications in Soil Science and Plant Analysis* 32: 921–950.

Batjes NH. 1996. Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science* 47: 151–163.

Beyers C de L. 1994. Die bemesting van aangeplante weidings. *Weidings = Pastures Elsenburg: Department of Agriculture*: 86–93.

Bhogal A, Chambers BJ, Nicholson FA. 2004. Relationships between fertiliser nitrogen additions, crop carbon returns and soil quality. In Hatch DJ, Chadwick DR, Jarvis SC, Roker JA (eds), *Controlling nitrogen flows and losses*. Wageningen Academic Publishers. pp 63–34

Bol R, Kandeler E, Amelung W, Glaser B, Marx MC, Preedy N, Lorenz K. 2003. Short-term effects of dairy slurry amendment on carbon sequestration and enzyme activities in a temperate grassland. *Soil Biology and Biochemistry* 35: 1411–1421.

Botha PR. 2003. Die produksiepotensiaal van oorgesaaide kikoejoeweiding in die gematigde kusgebied van die Suid-Kaap. PhD thesis, University of the Freestate, Bloemfontein, South Africa.

Brady NC, Weil RR. 2002. *The Nature and Properties of Soils* (13th edn). Prentice Hall

Cartes P, Jara AA, Demanet R, de la Luz Mora M. 2009. Urease activity and nitrogen mineralization kinetics as affected by temperature and urea Input rate in Southern Chilean Andisols. *Journal of Soil Science and Plant Nutrition* 9: 69–82.

Cataldo DA, Haroon H, Schrader LE, Young VL. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitrate of salicylic acid. *Communications in Soil Science and Plant Analysis* 6: 71–80.

Chang EH, Chung RS, Tsai YH. 2007. Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. *Soil Science and Plant Nutrition* 53: 132–140.

Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens

GE. 2009. Controlling Eutrophication: Nitrogen and Phosphorus. *Science* 323: 1014–1015.

Cookson WR, Marschner P, Clark IM, Milton N, Smirk MN, Murphy DV, Osman M, Stockdale EA, Hirsch PR. 2006. The influence of season, agricultural management, and soil properties on gross nitrogen transformations and bacterial community structure. *Australian Journal of Soil Research* 44: 453–465.

Dessureault-Rompré J, Zebarth BJ, Burton DL, Sharifi M, Cooper J, Grant CA, Drury CF. 2010. Relationships among Mineralizable Soil Nitrogen, Soil Properties, and Climatic Indices. *Soil Science Society of America Journal* 74: 1218–1227.

Dolan MS, Clapp CE, Allmaras RR, Baker JM, Molina JAE. 2006. Soil organic carbon and nitrogen in a Minnesota soil as related to tillage, residue and nitrogen management. *Soil and Tillage Research* 89: 221–231.

DWAF (Department of Water Affairs and Forestry). 1996. *South African Water Quality Guidelines: Volume 1 Domestic Water Use* (2nd edn). Pretoria.

Edenborn SL, Sexstone AJ, Sutanto Y, Chapman JA. 2011. Relationships among contrasting measurements of microbial dynamics in pasture and organic farm soils. *Applied and Environmental Soil Science* 2011: 1–10.

EEC. 1991. Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. *Official Journal L* 375: 1–8.

Fessehazion MK, Abraha AB, Everson CS, Truter WF, Annandale JG, Moodley M. 2012. Water Use and Nitrogen Application for Irrigation Management of Annual Ryegrass and Kikuyu Pasture Production. *Water Research Commission Report* No. TT 520/12

Fessehazion MK, Stirzaker RJ, Annandale JG, Everson CS. 2011. Improving nitrogen and irrigation water use efficiency through adaptive management: A case study using annual ryegrass. *Agriculture, Ecosystems and Environment* 141: 350–358.

Franzluebbers AJ, Stuedemann JA. 2009. Soil-profile organic carbon and total nitrogen during 12 years of pasture management in the Southern Piedmont USA. *Agriculture, Ecosystems and Environment* 129: 28–36.

Franzluebbers AJ, Stuedemann JA, Schomberg HH, Wilkinson SR. 2000. Soil organic C and N pools under long-term pasture management in the Southern Piedmont USA*. Soil Biology and Biochemistry* 32: 469–478.

Fulkerson WJ, Lowe KF, Hume DE. 2011. Forages and Pastures | Perennial Forage and Pasture

Crops - Establishment and Maintenance. In: Fuquay JW, Fox PF, McSweeney PLH (eds), *Encyclopedia of Dairy Science* (2nd edn). Academic Press. pp. 586–593.

Gianfreda L, Rao MA, Piotrowska A, Palumbo G, Colombo C. 2005. Soil enzyme activities as affected by anthropogenic alterations: Intensive agricultural practices and organic pollution. *Science of the Total Environment* 341:265–279.

Glass GV, Peckham PD, Sanders JR. 1972. Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. *Review of Educational Research* 42: 237-288.

Guntiñas ME, Leirós MC, Trasar-Cepeda C, Gil-Sotres F. 2012. Effects of moisture and temperature on net soil nitrogen mineralization: A laboratory study. *European Journal of Soil Biology* 48: 73–80.

Hadas A, Kautsky L, Goek M, Kara EE. 2004. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. *Soil Biology and Biochemistry* 36: 255–266.

Halvorson AD, Wienhold BJ, Black AL. 2002. Tillage, Nitrogen, and Cropping System Effects on Soil Carbon Sequestration. *Soil Science Society of America Journal* 66: 906.

Hart SC, Nason GE, Myrold DD, Perry DA. 1994. Dynamics of Gross Nitrogen Transformations in an Old-Growth Forest : The Carbon Connection. *Ecology* 75: 880–891.

Hatch DJ, Lovell RD, Antil RS, Jarvis SC, Owen PM. 2000. Nitrogen mineralization and microbial activity in permanent pastures amended with nitrogen fertilizer or dung. *Biology and Fertility of Soils* 30: 288–293.

IUSS Working Group WRB. 2015. World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. *World Soil Resources Reports No*. 106. FAO, Rome

Kandeler E, Gerber H. 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils* 6: 68–72.

Keeney DR, Miller RH, Page AL. 1982. Chemical and microbiological properties. In: Page AL, Keeney DR, Miller RH (eds), Methods of soil analysis. Part 2: Chemical and microbiological properties (2nd edn). Agronomy Series no. 9. Madison: American Society of Agronomy.

Khan SA, Mulvaney RL, Ellsworth TR, Boast CW. 2007. The Myth of Nitrogen Fertilization for Soil Carbon Sequestration. *Journal of Environment Quality* 36: 1821–1832.

Kotzé E, Sandhage-Hofmann A, Amelung W, Oomen RJ, du Preez CC. 2017. Soil microbial communities in different rangeland management systems of a sandy savanna and clayey grassland ecosystem, South Africa. *Nutrient Cycling in Agroecosystem*. 107(2): 227-245.

Ledgard SF, Penno JW, Sprosen MS. 1999. Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. *Journal of Agricultural Science* 132: 215–225.

Liu E, Yan C, Mei X, He W, Bing SH, Ding L, Liu Q, Liu S, Fan T. 2010. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. *Geoderma* 158: 173–180.

Marais JP. 2001. Factors affecting the nutritive value of kikuyu grass. *Tropical Grasslands* 35: 65–84.

Matlou MC, Haynes RJ. 2006. Soluble organic matter and microbial biomass C and N in soils under pasture and arable management and the leaching of organic C, N and nitrate in a lysimeter study. *Applied Soil Ecology* 34: 160–167.

McKenzie FR, Tainton NM. 1993. Pattern of volatilized nitrogen loss from dryland kikuyu pastures after fertilization. *African Journal of Range and Forage Science* 10: 86–91.

Miles N. 1997. Responses of productive and unproductive kikuyu pastures to top - dressed nitrogen and phosphorus fertiliser. *African Journal of Range and Forage Science* 14: 1–6.

Murphy DV, Stockdale EA, Hoyle FC, Smith JU, Fillery IRP, Milton N, Cookson WR, Brussaard L, Jones DL. 2004. Matching supply with demand. In Hatch DJ, Chadwick DR, Jarvis SC, Roker JA (eds), *Controlling nitrogen flows and losses*. Wageningen Academic Publishers. pp. 101–112

Nelson DW, Sommers LE. 1982. Total carbon, organic carbon, and organic matter. In: Page AL (ed), *Methods of soil analysis: Part 2, Chemical and microbiological properties.* American Society of Agronomy, Madison, Wisconsin, pp. 539-579.

Pascual JA, García C, Hernandez T. 1999. Lasting microbiological and biochemical effects of the addition of municipal solid waste to an arid soil. *Biology and Fertility of Soils* 30: 1–6.

Pembleton KG, Rawnsley RP, Burkitt LL. 2013. Environmental influences on optimum nitrogen fertiliser rates for temperate dairy pastures. *European Journal of Agronomy* 45: 132–141.

Roscoe R, Vasconcellos CA, Furtini-Neto AE, Guedes GAA, Fernandes LA. 2000. Urease activity and its relation to soil organic matter, microbial biomass nitrogen and urea-nitrogen assimilation by maize in a Brazilian Oxisol under no-tillage and tillage systems. *Biology and Fertility of Soils* 32: 52–59.

Ross DJ, Newton PC, Tate KR. 2004. Elevated [CO_2] effects on herbage production and soil carbon and nitrogen pools and mineralization in a species-rich, grazed pasture on a seasonally dry sand. *Plant and Soil* 260: 183–196.

Ruz-Jerez BE, White RE, Ball PR. 1994. Long-term measurement of denitrification in three contrasting pastures grazed by sheep. *Soil Biology Biochemistry* 26(1):29-39.

Saha S, Prakash V, Kundu S, Kumar N, Mina BL. 2008. Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean-wheat system in N-W Himalaya. *European Journal of Soil Biology* 44: 309–315.

Schipper LA, Percival HJ, Sparling GP. 2004. An approach for estimating when soils will reach maximum nitrogen storage. *Soil Use and Management* 20: 281–286.

Sharifi M, Zebarth BJ, Burton DL, Grant CA, Cooper JM. 2007. Evaluation of Some Indices of Potentially Mineralizable Nitrogen in Soil. *Soil Science Society of America Journal* 71: 1233–1239.

Simić A, Vučković S, Sokolović D, Stanisavljević R, Mandić V, Duronić G. 2009. Changes of Crude Protein Content in Italian Ryegrass Influenced By Spring Nitrogen Application. *Biotechnology in Animal Husbandry* 25: 1171–1179.

Snedecor GW, Cochran WG. 1980. Statistical methods, 7th edn. Ames, Iowa State University Press, pp 507.

Sparling G, Lilburne L, Vojvodic-Vukovic M. 2008. Provisional Targets for Soil Quality Indicators in New Zealand. *Landcare Research Scinece Series No. 34*. Manaaki Whenua Press. Palmerston North, N.Z.

Stirzaker RJ. 2003. When to turn the water off: Scheduling micro-irrigation with a wetting front detector. *Irrigation Science* 22: 177–185.

Stout DG, Brooke B, Hall JW, Thompson DJ. 1997. Forage yield and quality from intercropped barley, annual ryegrass and different annual legumes. *Grass and Forage Science* 52: 298–308.

Swanepoel PA, Botha PR, du Preez CC, Snyman HA, Labuschagne J. 2015a. Managing cultivated pastures for improving soil quality in South Africa: challenges and opportunities. *African Journal of Range and Forage Science* 32: 91–96.

Swanepoel PA, du Preez CC, Botha PR, Snyman HA. 2015b. A critical view on the soil fertility status of minimum-till kikuyu–ryegrass pastures in South Africa. *African Journal of Range and Forage Science* 32: 113–124.

Swanepoel PA, Botha PR, Snyman HA, Preez CC. 2014a. Impact of cultivation method on productivity and botanical composition of a kikuyu-ryegrass pasture. *African Journal of Range and Forage Science* 31: 215–220.

Swanepoel PA, Habig J, Preez CC, Botha PR, Snyman HA. 2014b. Biological quality of a podzolic soil after 19 years of irrigated minimum-till kikuyu-ryegrass pasture. *Soil Research* 52: 64–75.

Swanepoel PA, du Preez CC, Botha PR, Snyman HA, Habig J. 2014c. Soil quality characteristics of kikuyu-ryegrass pastures in South Africa. *Geoderma* 232–234: 589–599.

Tainton NM. 2000. *Pasture Management in South Africa*. University of Natal Press. Pietermaritzburg, South Africa.

TIBCO Software. 2017. Statistica (data analysis software system), version 13. TIBCO Softw., Palo Alto, CA.

Thompson RB, Fillery IRP. 1998. Fate of urea nitrogen in sheep urine applied to soil at different times of the year in the pasture–wheat rotation in south Western Australia. *Australian Journal of Agricultural Research* 49: 495–510.

van der Colf J. 2011. The production potential of Kikuyu (*Pennisetum clandestinum*) pastures over-sown with Ryegrass (*Lolium* spp.). MSc(Agric) thesis, University of Pretoria, Pretoria, South Africa.

Vogeler I, Lucci G, Shepherd M. 2016. An assessment of the effects of fertilizer nitrogen management on nitrate leaching risk from grazed dairy pasture. *Journal of Agricultural Science* 154: 407–424.

Wang C, Wan S, Xing X, Zhang L, Han X. 2006. Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. *Soil Biology and Biochemistry* 38: 1101–1110.

Wright AF, Bailey JS. 2001. Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. *Communications in Soil Science and Plant Analysis* 32(19-20): 3243-3258.

Zaman M, Cameron KC, Di HJ, Inubushi K. 2002. Navigating wall-sized displays with the gaze: A proposal for cultural heritage. Nutrient Cycling in *Agroecosystems* 63: 275–290.

Zhang X, Wang Q, Gilliam FS, Bai W, Han X, Li L. 2012. Effect of nitrogen fertilization on net nitrogen mineralization in a grassland soil, northern China. *Grass and Forage Science* 67: 219–230.

Chapter 4 The effect of N rates on pasture herbage production, forage quality and botanical composition

4.1 Introduction

Kikuyu (*Pennisetum clandestinum*) is used as a pasture base in the dairy producing area of the southern Cape and when focussing on the fodder flow program, species with a higher forage quality, such as annual ryegrass (*Lolium multiflorum*) and clover (*Trifolium* spp.) are sown into (Harris and Bartholomew 1991; Botha 2003; Garcia et al. 2008; Sinclair and Beale 2010; van der Colf et al. 2015a). With proper management, adequate rainfall and nitrogen (N) fertilisation, kikuyu can be a highly productive summer pasture species although some anti-quality factors exist (Cross 1979). These anti-quality factors [mineral imbalances, nitrate accumulation, oxalate (Marais 2001)], alongside a low forage quality compared to temperate pasture species, are not desirable pasture characteristics for high producing dairy cows. This, however, can be overcome by feeding concentrates in order to prevent a decrease in milk production (Garcia et al. 2014).

Besides inorganic fertilisers, N input into the pasture systems may be in the form of *inter alia*, biological N fixation through legumes, soil N mineralisation and animal excreta (Peoples and Baldock 2001; Cassman et al. 2002). Incorporating legumes to supply the system with N through fixation is often not successful. Reasons include that clovers do not compete well with grasses due to overshadowing as a result the length of grasses at grazing (Botha 2003). The benefit of N fixation from clovers is often not enough to support growing grasses, resulting in additional inorganic N input, which in turn has a negative effect on the persistence of clovers. For these reasons, producers commonly establish pastures as pure-grass swards. These producers then rely on a high N application rates of inorganic fertilisers to increase yield (Garcia et al. 2014). For kikuyu-based systems in particular, fertiliser guidelines were developed from small plot trials associated with deep tillage and without correction from N inputs via manure and urine (Beyers 1994). Current N fertiliser guidelines for kikuyu-ryegrass pasture are 300 to 500 kg N ha^{-1} $year^{-1}$ (Marais 2001). Such high rates not only makes N fertilisation one of the most expensive inputs in these systems (Baligar et al. 2001; Masclaux-Daubresse et al. 2010), but the pasture systems are potentially also over-fertilised, especially during winter when nitrate leaching risk is high, which may be detrimental to the environment (Elliott and Abbott 2003; Pembleton et al. 2013). Supplying N when it is not utilised by plants is a financial waste and losses can be expected through eutrophication, denitrification and ammonia volatilisation (Ledgard et al. 1999; Canfield et al. 2010). To prevent losses, it is important to consider the adoption of strategic N application regimes. Various authors have already reported on the potential to only apply N in the growing seasons of kikuyu and ryegrass (Marais 1980, 1990; Eckard et al. 1995; Fulkerson et al. 1998; Pembleton et al. 2013). The aim of this study was to reassess N fertiliser guidelines of kikuyu and kikuyu-ryegrass pastures in the southern Cape under a minimum-tillage regime and grazing, aimed at optimising the quality of pasture, measured by CP, while still maintaining yield. Developing a strategic N fertiliser programme may aid in preventing environmental and financial losses since agronomic N use efficiency (ANUE) is taken into account.

4.2 Materials and Methods

4.2.1 Experimental site characterisation

The study was conducted over a period of two years, April 2016 to March 2018, on the Outeniqua Research Farm near George in the Western Cape Province, South Africa. The trials were performed on a podzol soil, specifically characterised as a Witfontein soil form. Both sites were characterised by long-term established kikuyu that had been under minimum-tillage management practices for more than ten years. The site is approximately 8 km from the Indian Ocean and situated at an altitude of 201 m (33 58’ 38” S, 22 25’ 16” E). Rain is received throughout the year with a total mean annual precipitation of 728 mm determined over long-term (35 years). The area has a temperate climate with minimum temperatures ranging between 7 and 15 °C and maximum temperatures between 18 and 25 °C, in winter and summer, respectively. For the purpose of this study, autumn months were defined as March, April and May; winter months as June, July and August; spring as September, October and November and the summer months as December, January and February.

4.2.2 Study design and treatments

For this study, two separate sites were used, one with a pure kikuyu sward and another where kikuyu was over-sown with annual ryegrass on an annual basis. Both sites were laid out as randomised block designs. Each site had six N fertilisation treatments replicated in four blocks. In total, there were 24 plots per site comprising an area of 15 × 15 m each. Five N treatments were applied at fixed rates (0, 20, 40, 60 and 80 kg N ha^{-1}), while the sixth treatment was applied strategically according to the nitrate concentration of the soil water after grazing. A detailed table describing the N rate applied per grazing cycle are presented in the Chapter 3 (Table 3.2).

4.2.2.1 Nitrate concentration determination in soil water

Soil water was obtained using FullStop™ Wetting Front Detectors planted at 150 mm and 300 mm depths. A Wetting front detector is a funnel-shaped passive lysimeter, buried at different depths in soil which can be applied for managing irrigation. When the soil potential around the WFD approaches -3 kPa, such as after an irrigation or rainfall event, free water is produced at the base of the funnel, which can then be extracted using a syringe. This water sample can be used to monitor soluble soil nutrients, such as nitrates, and salinity. As the water passes through a filter and collects at the reservoir, a float is magnetically activated showing that there is a water sample to be retrieved for analysis by using a syringe (Stirzaker 2003). During the study nitrate concentration in the soil water was determined by using a Horiba Scientific LAQUAtwin compact water quality meter, as well as Quantofix® nitrate test strips. This was done throughout the year whenever the WFD had collected enough water or the float was triggered. An average of the month's measurements obtained from the 150 mm WFD was used to calculate whether N should be applied and at what rate. When the average nitrate concentration of the month was above 50 mg L^{-1}, no N was applied to the pasture. A nitrate concentration of between 25 and 50 mg L^{-1} resulted in an N application of 25 kg N ha^{-1}. When the nitrate concentration was below 25 kg N ha^{-1}, 50 kg N ha^{-1} was applied (Fessehazion et al. 2011). The optimum nitrate level to maintain, at which both yield and forage quality of ryegrass is ideal, is considered to be 50 mg L^{-1} according to (Fessehazion et al. 2011). Based on the assumption that mixed pasture systems will behave similarly in the podzolic soil, the optimal nitrate concentration during the study was also set at 50 mg N L^{-1}. This standard, however, was adapted from September 2016 after it was observed that the Nvar plots had a low pasture production relative to other plots and showed visual signs of an N deficiency, irrespective of the fact that the N concentration in soil water indicated that no N fertiliser is required. As result, new nitrate concentrations limits were set. When the average nitrate concentration of the month was above 75 mg L^{-1}, no N were applied to the pasture. When the concentrations were between 50 and 75 mg L^{-1}, 25 kg N ha^{-1} was applied. When the concentrations were below 50 mg L^{-1}, 50 kg N ha^{-1} was applied. The ranges of N applied per plot regarding Nvar treatments can be found in Table 3.3. When the four Nvar replicates are averaged within a season, the N applications were applied within the particular season with the particular amount that can be found in Table 4.1.

Table 4.1: Treatment Nvar nitrogen (N) applications (kg N ha^{-1} $season^{-1}$) for each season during year one (1) and year two (2), when individual replicates were averaged within a specific season for the kikuyu and kikuyu-ryegrass site

Season	Kikuyu	Kikuyu-ryegrass
Winter 1	10.00	10.00
Spring 1	25.00	0.00
Summer 1	18.75	12.50
Autumn 1	12.50	0.00
Winter 2	25.00	6.25
Spring 2	12.50	6.25
Summer 2	0.00	0.00

Nvar = varying N rate according to nitrate concentration in the soil water

4.2.3 Pasture management

The kikuyu and kikuyu-ryegrass sites were managed and investigated separately following grazing by Jersey cows to a target post-grazing height of 50 mm. In addition, the kikuyu site was slashed during autumn 2016 and again in 2017 to a 50 mm height to prevent a build-up of a fibrous mat.

The kikuyu-ryegrass site was prepared by over-sowing annual ryegrass into an existing, long-term kikuyu-base in April 2016, and again in March 2017. A germination test was done on the annual ryegrass (cv. Barmultra II) seed prior to planting (ISTA 2016). Before the existing kikuyu was mulched to ground level in preparation for planting, using a Nobili mulcher, it was grazed down to 50 mm. Using an Aitchison seed-drill, annual ryegrass was then planted into kikuyu at 25 kg ha^{-1} (van der Colf 2011; Swanepoel et al. 2014). After planting, the area was rolled with a Cambridge-type roller. No N was applied at the time of over-sowing of ryegrass. This was done to prevent the kikuyu base from competing with the emerging ryegrass seedlings. Limestone ammonium nitrate (LAN) was used as N source during the study and was applied directly after grazing.

Herbage was allocated to dairy cows based on estimates of herbage-on-offer as determined by a rising plate meter. Linear regression curves and equations were used according to the season to estimate the number of cows required to graze the research area (van der Colf 2011), with approximately 5 kg dry matter (DM) herbage allocated per cow per grazing day. Grazing cycle was approximately 28 days during summer and 32 days during winter (van der Colf 2011). Cows were not allocated to specific treatments, but allowed to voluntarily graze over all plots. Thus, the plots were strip grazed by cows in order to equally distribute any carry-over effects that may have originated from either the concentrate fed in the milking parlour or previous pasture. A grazing cycle was defined as the period from one harvest to the next.

Permanent over-head sprinkler irrigation was used on both pastures. Sprinklers were spaced 15 m apart. Irrigation scheduling was based on tensiometer readings, with management aimed at maintaining a matrix potential between -25 and -10 kPa. One tensiometer was installed at a depth of 150 mm on each pasture site. Rain gauges were installed next to the tensiometers to measure the amount of water (rain and/or irrigation) received by the pasture, in addition to the Decagon Weather Station (Figure 3.1).

4.2.4 Sampling and analysis

Herbage sampling was done prior to grazing. Five rings (0.0985 m^2) were cut on each plot in order to determine yield to a height of 30 mm above ground. The cutting was done manually with a hand shear, with rings placed randomly within the plots. Samples were dried for 72 hours at 60°C to determine DM content and herbage yield (kg DM ha^{-1}). Total seasonal yields were calculated through the summation of the grazing cycle yields that fell within the specific season. A harvest was considered to fall within a specific season when more than half of the growing period occurred within that season.

An additional three rings (0.0985 m^2) were cut once per season per plot to determine botanical composition. Each site was fractioned into five classes, namely kikuyu, ryegrass, other grasses, legumes and weed components. Other grasses included species such as *Eragrostis plana*, *Paspalum notatum*, *Poa annua*, *Bromus catharticus* and *Sporobolus africana*. These other grass species were classified as such since it was not sown into the kikuyu pasture base. On the kikuyu site, ryegrass (volunteer) were classified separately because it made a major contribution to the pasture. Volunteer legumes in the pasture was mostly white clover (*Trifolium* spp.), while the species that contributed to the weed component were mostly broadleaf spp. with the exception of *Cyperus* spp. The other broadleaf weed species were *Arctotheca calendula, Taraxacum officinale, Stellaria media, Rumex crispus* and *Urtica* spp. Each fraction was weighed individually to obtain wet weight, placed in the oven in marked brown paper bags for 72 hours at 60°C. The dry weight was determined and used to calculate the proportional contribution of each fraction on a dry mass basis.

Five dried pasture samples per plot were pooled and milled (SWC Hammer mill, 1 mm sieve) to determine crude protein content. Total N in the herbage was determined with the Kjeldahl method and a factor of 6.25 was used to determine crude protein content from this value (AOAC 2000).

The ANUE was used to calculate the expected production increase due to applied N per hectare and expressed as kg DM kg^{-1} N ha^{-1}. It was calculated by subtracting the herbage production of the control from that of the treatment and dividing the answer by the amount of N applied in the treatment as per the following formula:

$$ANUE = \frac{Pasture\ production\ (fertilized) - pasture\ production\ (control)}{N\ supply} = kg\ DM\ kg^{-1}\ N\ ha^{-1}$$

4.2.5 Statistical analyses

The Restricted Maximum Likelihood (REML) method was used to test for differences between treatments. Treatment, time and their interaction were specified as the fixed effects in order to take the repeated measures into account. Block was specified as a random effect. The means were separated using Fishers' Protected Least Significant Difference (LSD) test at a 5% significance level (Glass et al. 1972; Snedecor and Cochran 1980*).* Data was analysed by the STATISTICA version 13.2 (TIBCO Software 2017). Furthermore, for a better overall interpretation of botanical composition, these components were analysed using non-metric multidimensional scaling. The Bray-Curtis/Whittaker dissimilarity matrix have been used to visualise how dissimilar the botanical composition were. The statistical programme R (version 3.3.2, package vegan and function monoMDS) was used for this analysis. The appropriate number of axes for each ordination was determined using a scree plot of ordination stress using the function dimcheckMDS. An ordination stress of less than 0.2 was maintained to ensure that there is not more dimensions added that does not give extra information with regards to season and N application rate.

4.3 Results and Discussion

4.3.1 Kikuyu pasture site

4.3.1.1.1 Herbage production per grazing cycle

The response of herbage production to N treatment was similar ($P>0.05$) in all grazing cycles (Table 4.2). The average herbage production per treatment per grazing cycle, calculated from May 2016 to March 2018, showed that N20, N40, N60 and N80 had a higher ($P\leq0.05$) herbage production than N0 and Nvar (Figure 4.1).

During November 2016 and February 2017, the herbage production, when data was averaged over treatments, was the highest ($P\leq0.05$) compared to all other grazing cycles (Figure 4.2). The November grazing cycle was long (41 days – not recommended for spring production) as this was when the harvest fit in best regarding the other studies on the research farm. The long grazing cycle could be the reason for the high production recorded during this month. To address the differences in the length of grazing cycles, growth rate per day (kg DM ha^{-1} day^{-1}) was calculated and there were no interaction ($P>0.05$) between treatments and grazing cycle (Table 4.2). Figure 4.3 confirms that the length of the grazing cycle was indeed the cause for higher production during November 2016, since it did not have the highest growth rate. The growth rate in the study varied from as high as 97 kg DM ha^{-1} day^{-1} (January 2017) to as low as 31 kg DM ha^{-1} day^{-1} (July 2017). From May 2016 to August 2016 and April 2017 to August 2017, a decrease in production was observed ($P\leq0.05$) (Figure 4.2). This is in agreement with previous findings that kikuyu becomes dormant during the late-autumn to early-spring (May to September) period (Marais 2001), resulting in lowered production. Irrespective of this, some herbage was still produced during these months. This was attributed to the slow growing and thus non-competitive kikuyu allowing other grasses, such as volunteer ryegrass and *Bromus* spp., to grow in the dormant months. This observation will be discussed under the botanical composition section.

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Table 4.2: ANOVA of kikuyu site (Num DF = Numerator degrees of freedom, Den. DF = Denominator degrees of freedom)

	Num. DF	Den. DF	F	P-value
Herbage production				
Treatment	5	18	5.33	0.004
Grazing cycle	21	378	44.34	<0.001
Treatment* Grazing cycle	105	378	0.59	0.999
Growth rate				
Treatment	5	18	6.06	0.002
Grazing cycle	20	360	74.43	<0.001
Treatment* Grazing cycle	100	360	0.77	0.943
Dry matter content				
Treatment	5	18	6.85	0.001
Grazing cycle	21	378	85.54	<0.001
Treatment* Grazing cycle	105	378	1.16	0.158
Herbage production				
Treatment	5	18	6.26	0.002
Season	7	126	352.34	<0.001
Treatment* Season	35	126	0.69	0.893
Pre-grazing Disc meter				
Treatment	5	18	5.73	0.002
Season	7	126	91.24	<0.001
Treatment* Season	35	126	0.63	0.942
Growth rate				
Treatment	5	18	6.63	0.001
Season	7	126	165.73	<0.001
Treatment* Season	35	126	0.66	0.920
Dry matter content				
Treatment	5	18	4.59	0.007
Season	7	126	40.97	<0.001
Treatment* Season	35	126	1.08	0.373
Post-grazing Disc meter				
Treatment	5	18	0.55	0.736
Season	7	126	34.24	<0.001
Treatment* Season	35	126	0.57	0.970
Botanical composition: Kikuyu				
Treatment	5	18	1.77	0.171
Season	7	126	82.16	<0.001
Treatment* Season	35	126	1.57	0.038
Botanical composition: Volunteer Ryegrass				
Treatment	5	18	5.91	0.002
Season	7	126	94.77	<0.001
Treatment* Season	35	126	1.48	0.060
Botanical composition: Volunteer Legumes				
Treatment	5	18	7.76	<0.001
Season	7	126	4.05	<0.001
Treatment* Season	35	126	2.28	<0.001
Agronomic N use efficiency				
Treatment	3	12	2.47	0.112
Season	7	84	0.61	0.744
Treatment* Season	21	84	0.40	0.990
Crude Protein				
Treatment	5	18	11.44	<0.001
Season	4	72	117.23	<0.001
Treatment* Season	20	72	6.72	<0.001

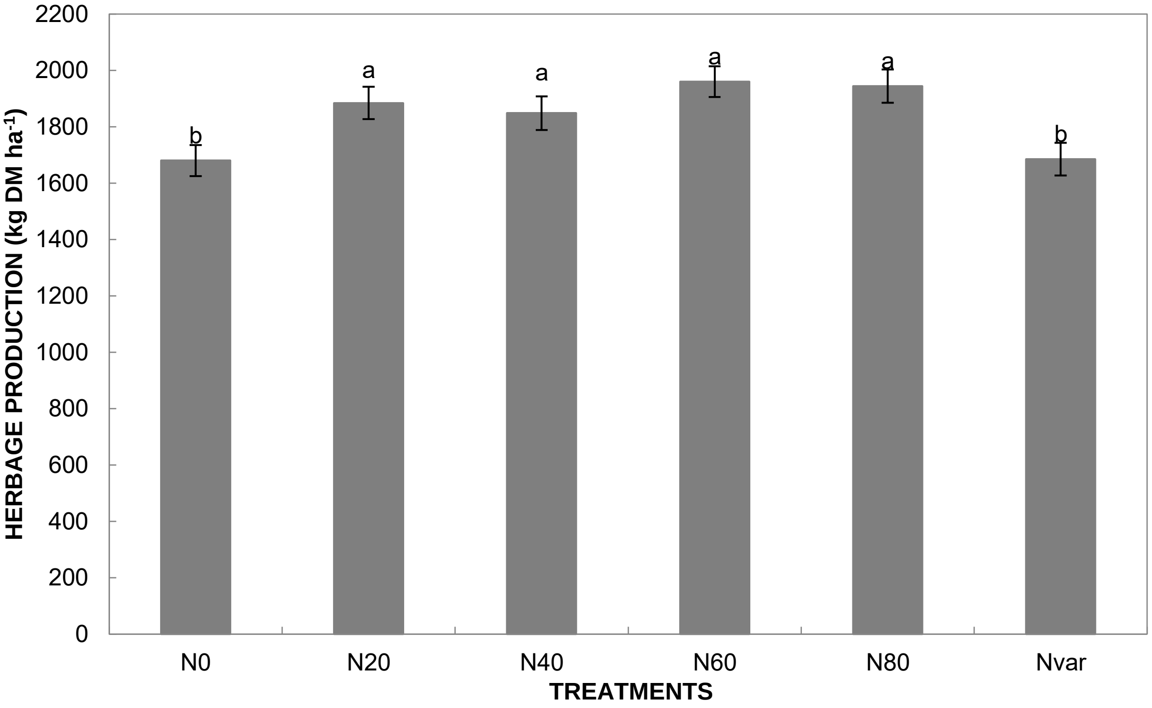

Figure 4.1: Average herbage production (kg DM ha^{-1}) of kikuyu site when averaged over grazing cycles from May 2016 to March 2018, as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar = varying N rate according to nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicate significant difference at a 5% level

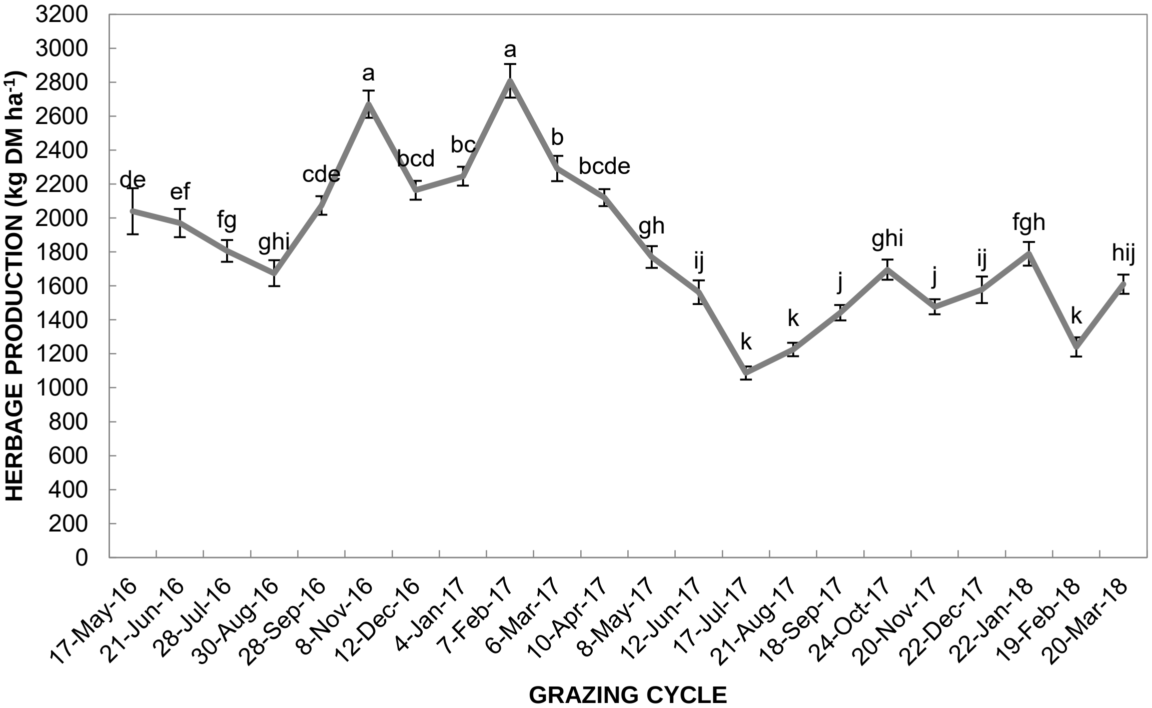

Figure 4.2: Average pasture herbage production (kg DM ha^{-1}) of the kikuyu site when averaged over treatments during the different grazing cycles of the study period. Error bars indicate standard error. No common letter above data points indicatesignificant difference at 5% level

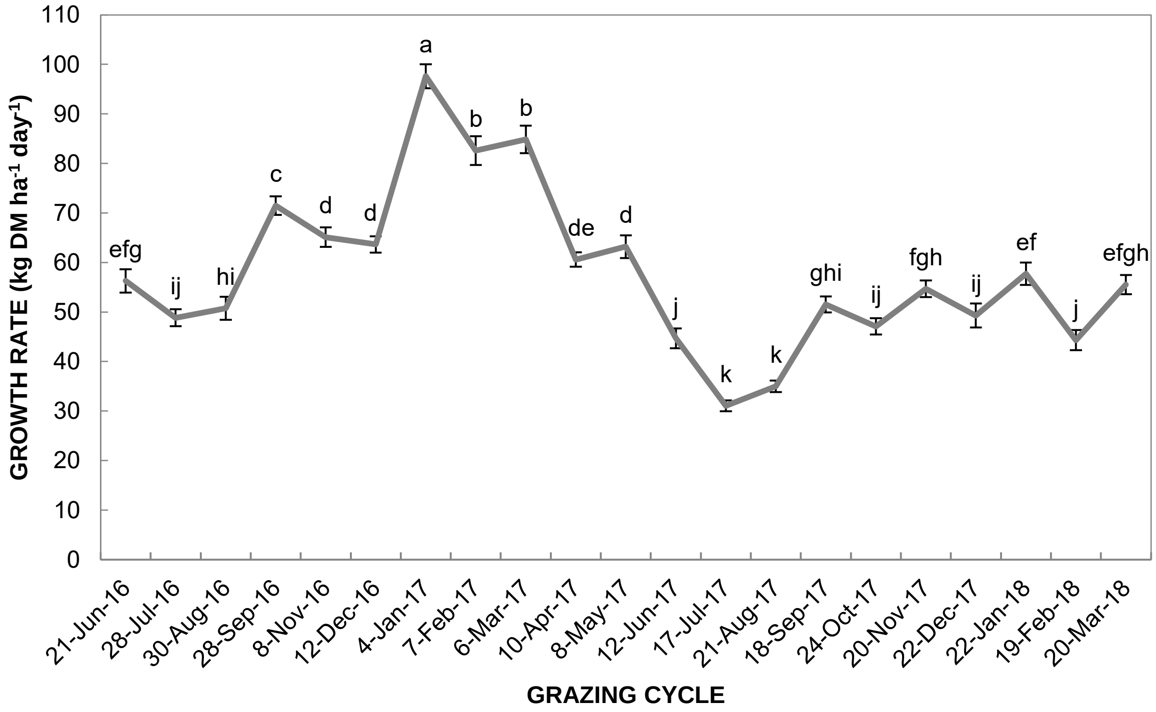

Figure 4.3: Mean pasture growth rate (kg DM ha^{-1} day^{-1}) of the kikuyu site when averaged over treatments during the different grazing cycles of the study period. Error bars indicate standard error. No common letter above data points indicatesignificant difference at 5% level

4.3.1.1.2 Seasonal herbage production

There were no interaction (P>0.05) between treatments and seasons with regards to seasonal herbage production for the kikuyu site (Table 4.2). Seasonal production, as affected by treatments, is presented in Figure 4.4. During winter of year one, treatment N60 and N80 produced more (P≤0.05) herbage compared to N0 and Nvar, but similar (P>0.05) to N20 and N40. During spring of the first year, treatment N40 had a higher (P≤0.05) herbage production than Nvar, but similar (P>0.05) to all other treatments. During summer of year one, treatment N80 had a higher (P≤0.05) herbage production than N0, N40 and Nvar, but similar (P>0.05) to N20 and N60. During autumn, treatment N60 produced higher (P≤0.05) than N0, Nvar, N20 and N40, but similar (P>0.05) to N80 in the first year. During winter of the second year, there were no treatment differences (P>0.05) and production was lower (P≤0.05) than during the other seasons. This was as result of one less grazing cycle occurring during this season (winter 2) than during the first winter (winter 1). Botanical composition may also have contributed to the production differences, but this will be discussed in more detail in section 4.3.1.2 . Reasons for the lower production of the N40 treatment compared to N20 in winter and summer of year one, although not significant (P>0.05), are not clear and warrants further investigation. During spring and summer of year two, there were no treatment differences (P>0.05; Figure 4.4).

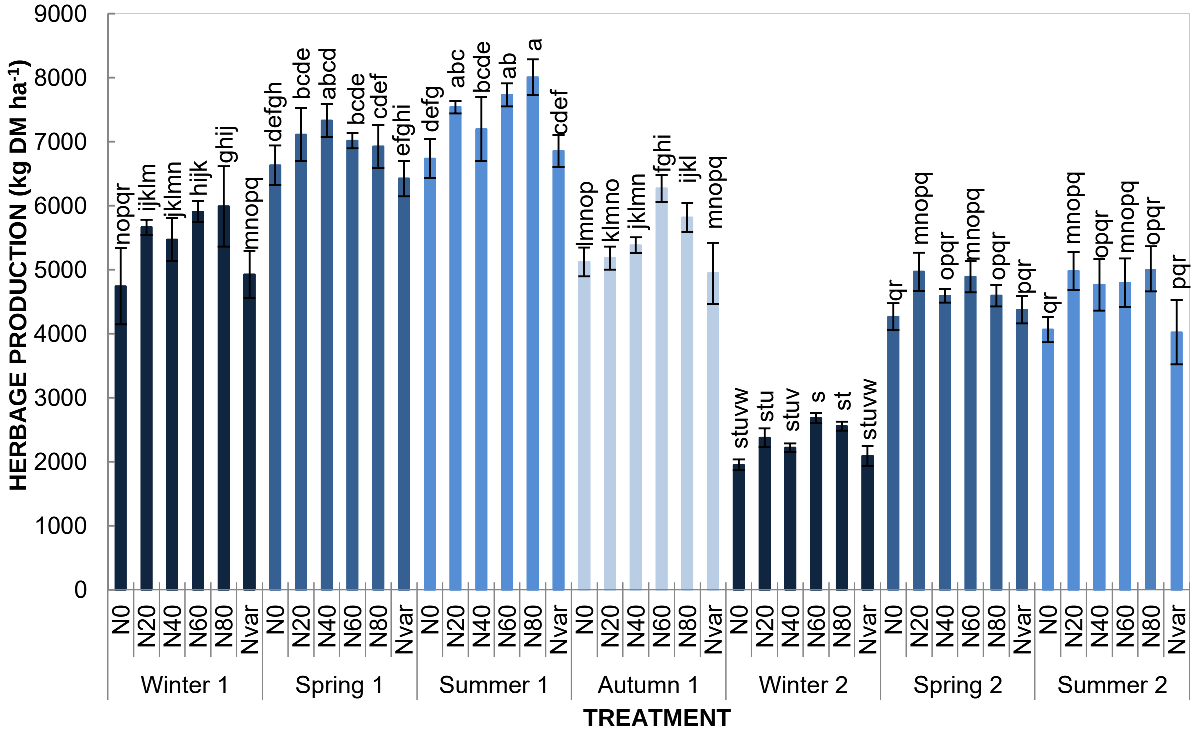

Figure 4.4: Seasonal herbage production (kg DM ha^{-1}) of the kikuyu site in seasons during year one (1) and two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$ and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

Overall, the production of this kikuyu site was higher during the first production year than what is reported in literature. On the same research location, seasonal yields were found to be 1.53, 6.29, 7.47 and 6 t DM ha^{-1} for winter, spring, summer and autumn, respectively, when pastures received a monthly N rate of 32 kg N ha^{-1} (Swanepoel et al. 2014). During the second production year, however, winter herbage production was still slightly higher at 2.31 t DM ha^{-1} while the spring and summer production of 4.61 t DM ha^{-1} that was recorded for both seasons during this study, was lower than that recorded by Swanepoel et al. (2014). The combined autumn and winter herbage production was approximately 5.2 t DM ha^{-1} in Australia under similar management practices and a N application rate of 322 kg N ha^{-1} $year^{-1}$ (autumn and summer received monthly applications, while spring received only two) (Fulkerson et al. 1999). This is lower compared to that of the current study where winter and autumn of year one, each had production in the range of 4.7 and 6.2 t DM ha^{-1} $season^{-1}$. Fulkerson et al. (1999) found approximate herbage production during summer to be 5.2 t DM ha^{-1} and during spring as 3.7 t DM ha^{-1}. In the current study, both spring and summer of year two had production in the range of 4 to 5 t DM ha^{-1} $season^{-1}$, while year one had higher production in the range of 6.5 to 8 t DM ha^{-1} $seaon^{-1}$.

Pre-grazing disc meter readings were taken on this site to estimate herbage production (Table 4.3). The regression equations that were used, however, were developed for kikuyu over-sown with Italian ryegrass (van der Colf 2011), since there are no existing equations for pure kikuyu pastures. This could be why the estimated herbage production as determined from disc meter readings was much lower than the measured production. The yields determined for Italian ryegrass-kikuyu during the same study were, however, also lower than the values determined here, further indicating that overall, the regressions and pasture type were not well suited to each other.

When the seasonal herbage production is considered as the main criteria the optimal mean recommended N fertiliser rate could be as low as 20 kg N ha^{-1}, or as high as 60 kg N ha^{-1} per grazing cycle, dependant on the particular season. To refine this broad optimal fertilisation rate, a strategic fertilisation, according to season, which will involve applying different N rates in different seasons, may result in improved profitability and also aid in environmental sustainability. The strategic fertilisation of kikuyu has, however, up to date received limited attention in the literature compared to kikuyu over-sown with temperate species.

Table 4.3: Cumulative seasonal herbage production (t DM ha^{-1}) available of year one (1) and two (2), estimated by pre-grazing disc meter readings on kikuyu pasture for treatments N0, N20, N40, N60, N80 and Nvar. No common letter below the values indicate significant difference at 5% level

	Winter 1	Spring 1	Summer 1	Autumn 1	Winter 2	Spring 2	Summer 2
N0	3.59 defghijk	2.80 jlmn	3.84 cdef	2.95 hijklm	1.54 qrs	3.11 ghijkl	3.07 ghijkl
N20	3.42 fghijk	3.48 fghijk	4.35 abcde	3.48 fghijk	2.02 nopq	3.85 cdefg	3.85 cdefg
N40	3.45 fghijk	3.37 fghijk	4.57 abc	3.40 fghijk	1.96 opqr	3.75 defgh	3.98 abcdef
N60	4.03 bcdef	3.60 defghji	4.78 a	3.56 defghijk	2.18 mnopq	4.11 abcdef	3.97 bcdef
N80	3.44 fghijk	3.60 efghij	4.70 ab	3.61 efghij	2.37 lmno	4.00 abcdef	4.33 abcd
Nvar	2.37 lmnop	2.46 lmno	3.94 bcdef	2.87 ijklm	1.46 qrs	2.79 klmn	2.94 ijklm

N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} and Nvar = varying N rate according nitrate concentration in the soil water.

Slow growing citrus trees have been found to have a lower N requirement compared to fast growing trees (Syvertsen and Smith 1996), and the same principal may be applied to other crops (Cassman et al. 2002). Assuming this is also the case in a pure kikuyu grass stand, higher N rates should be applied during seasons of high production, while in seasons when production is relatively low, lower N rates should be applied. The response of seasonal growth rate to treatment were similar ($P>0.05$) in all seasons (Table 4.2). The effects of season and treatment on the seasonal growth rate are available in Figure 4.5. There were no treatment differences ($P>0.05$) during spring of both year one and two. During winter of year one, treatment N60 and N80 had higher ($P\leq0.05$) seasonal growth rates compared to N0 and Nvar, but similar ($P>0.05$) to N20 and N40. With regards to winter of the second year, treatment N60 had a higher ($P\leq0.05$) seasonal growth rate compared to N0, but similar ($P>0.05$) to the other treatments. During summer of year one, treatment N80 had a higher ($P\leq0.05$) seasonal growth rate compared to N0, Nvar and N40, but similar (P>0.05) to N20 and N60. During the second year, summer seasonal growth rates of treatments N20 and N80 was higher (P≤0.05) compared to N0 and Nvar, but similar (P>0.05) to N40 and N60. Autumn seasonal growth rates were the highest (P≤0.05) in treatment N60 compared to all the other treatments, with the exception of N80 to which N60 was similar (P>0.05). These results confirm that seasonal fertiliser rates should be adjusted to the growth that can be expected during a specific season. The same strategy as the slow growing citrus trees, was followed by Fulkerson et al. (1999) by applying N at monthly intervals during summer and autumn when kikuyu growth is high, but only twice during spring when kikuyu growth is lower.

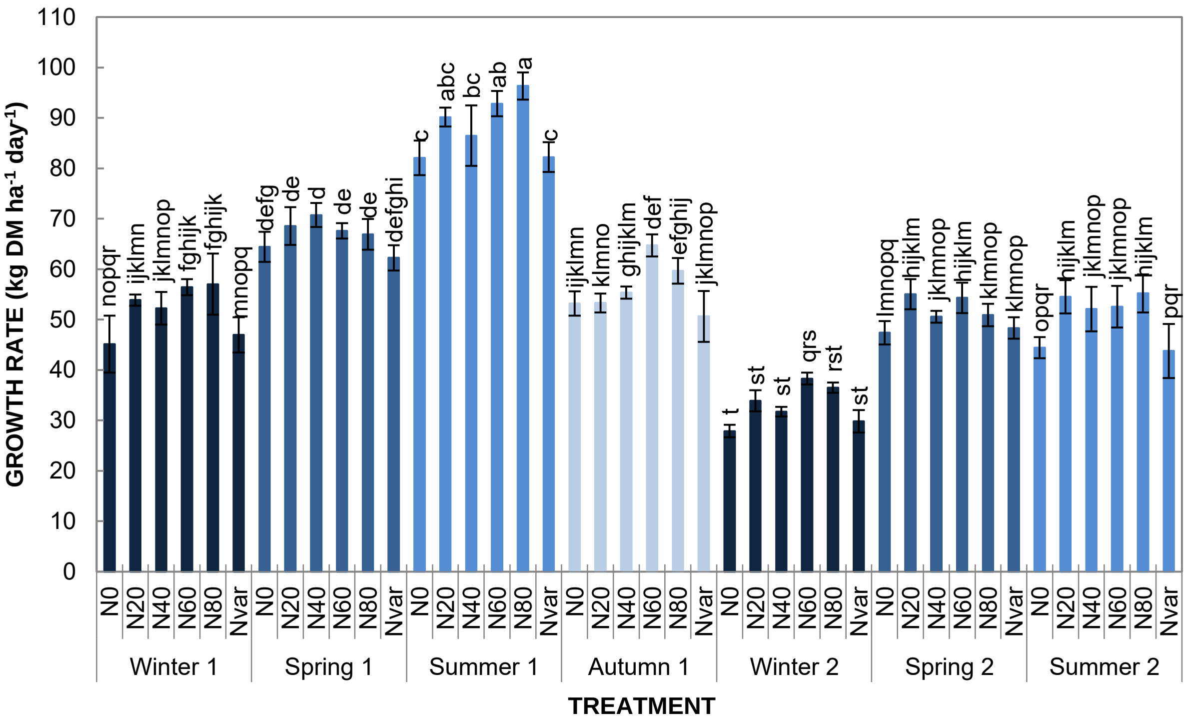

Figure 4.5: Average seasonal growth rate (kg DM ha^{-1} day^{-1}) of the kikuyu site in seasons during year one (1) and two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

4.3.1.1.3 Total annual dry matter production

The total annual dry matter production for year 1 (June 2016 to June 2017) ranged from 23153 to 26925 kg DM ha^{-1}, with treatment N60 and N80 producing higher (P≤0.05) than N0 and Nvar, but similar (P>0.05) to N20 and N40 (Figure 4.6). Annual herbage production of kikuyu generally ranges between 11.3 and 15 t DM ha^{-1} y^{-1}, when fertilised with 400 to 600 kg N ha^{-1} y^{-1} (Cook and Mulder 1984; Fulkerson et al. 1999; Lowe et al. 2011; Garcia et al. 2014). Pearson et al. (1985) reported total annual herbage production rates of 23.8, 15.9 and 14.8 t DM ha^{-1} when kikuyu received 120 kg N ha^{-1} after each harvest. They ascribed these differences in production to different locations based at different degrees of latitude. The highest production of 23.8 t DM ha^{-1} year^{-1} with N application rate of 120 kg N ha^{-1} after every harvest was similar to the N0 treatment in the current trial. The latitude at 34 °S (similar to the current study) had a production of between 11 and 15 t DM ha^{-1}, much lower than the current study (Pearson et al. 1985). This production, however, was calculated from three N application rates and might have been skewed by the lower production at lower N rates, since they also reported that kikuyu responded linearly to an increased N application rate.

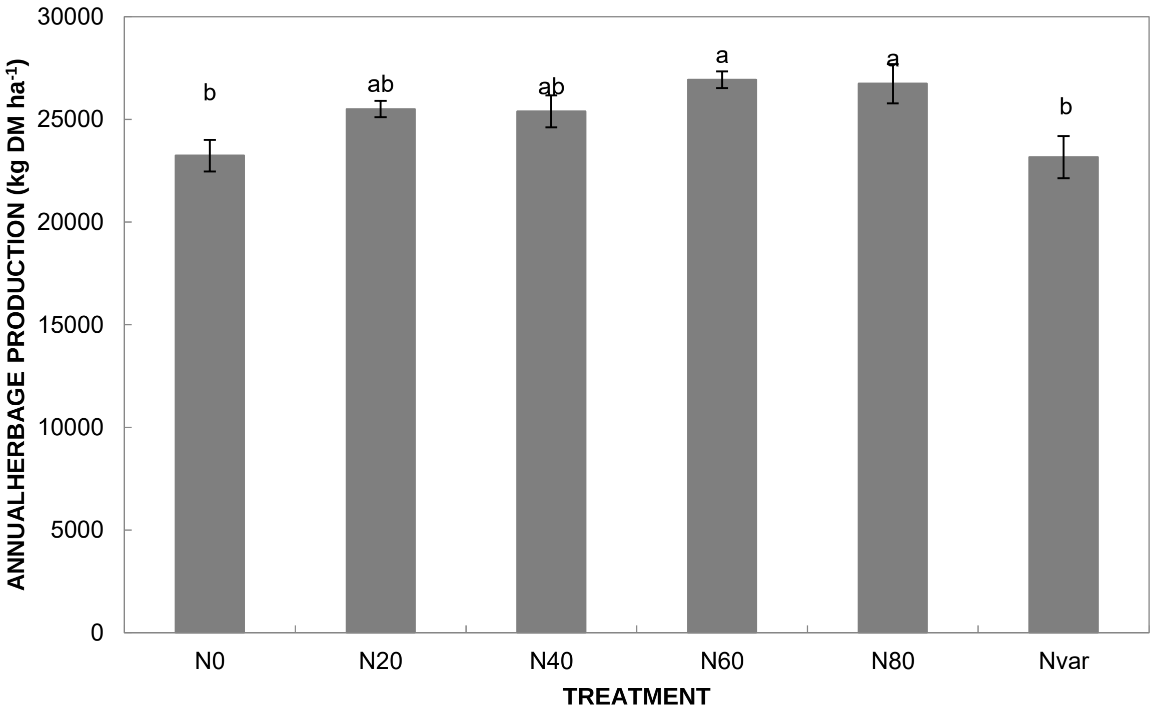

Figure 4.6: Annual herbage production (kg DM ha^{-1}) of the kikuyu site as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60 80 kg N ha^{-1} and Nvar = varying rate of N application according to soil water nitrate concentration. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

In trials that were conducted on a similar location as the current trial, annual yields of 13.8 t DM ha^{-1} (Botha et al. 2008a) and 21.29 t DM ha^{-1} (Swanepoel et al. 2014) were found for kikuyu pasture. As seen in the previous section on seasonal production, the major differences between the current study and that of Swanepoel et al. (2014), is the production during the winter season of year one. This agrees with findings from Botha et al. (2008a) that recorded high growth rates for summer and autumn and lower rates for spring, which further motivates the hypothesis that the difference in annual yield found in the current study due to herbage production differences in winter. Since management practices were consistent among these trials, the differences could also be ascribed to botanical composition in winter. This will be discussed in the botanical composition section hereafter.

Cross (1978) as cited in Dugmore (2011) reported a kikuyu herbage production of 15 to 16 t DM ha^{-1} for a N application rate of 300 kg N ha^{-1} year^{-1}, but also indicated that total annual production rates of up to 19 t DM ha^{-1} can be expected with the higher rainfall experienced at Cedara.

Similarly, Köster (1991) as cited in Dugmore (2011) found herbage production of 20.2 t DM ha^{-1} on irrigated kikuyu when N application rate was 230 kg N ha^{-1} during the growing season in Gauteng (University of Pretoria Experimental Farm).

Kikuyu builds up a fibrous mat material through time, and particularly following summer (high DM content) (van der Colf 2011). As the samples were cut within five rings placed randomly within the trial, material remaining from the previous grazing cycle might have been included, resulting in an overestimation of production. A high DM content is indicative of this (Figure 4.9). Residual material on the pasture ranged from 197.8 to 654.3 kg DM ha^{-1} above 30 mm according to rising plate meter readings. The season affected the post-grazing pasture height (Table 4.2) and the effect is seen in Figure 4.7, where summer and autumn of year one was higher ($P \leq 0.05$) in post-grazing height compared to the other season. This was due to kikuyu being an major contributing specie to the pasture during these seasons, as will be confirmed in section 4.3.1.2 . Calculating residual material post-grazing was done by using regression models for Italian ryegrass sown into kikuyu, since volunteer ryegrass contributed greatly to the pasture (discussed in section 4.3.1.2.2) (van der Colf 2011). There is however a model for calculating pre- and post-grazing for pure kikuyu pasture in winter, spring and summer (Botha 2003).

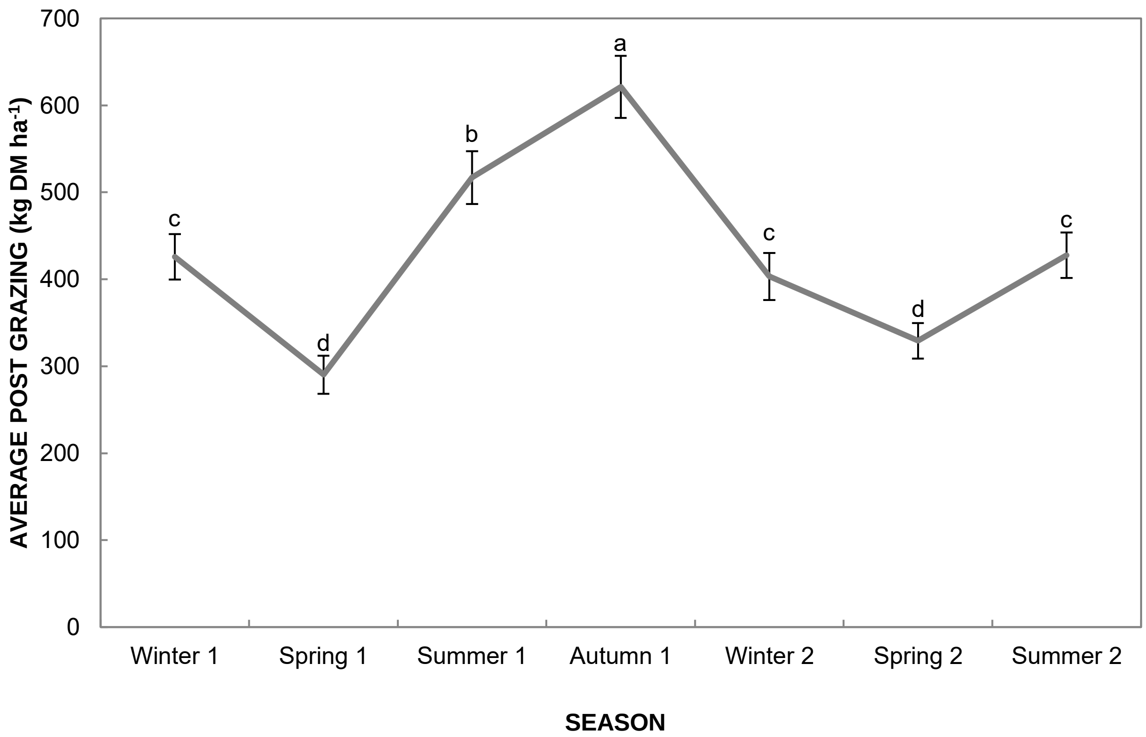

Figure 4.7: Average post-grazing (kg DM ha^{-1}) pasture yield of the kikuyu site as affected seasons during year one (1) and two (2). Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level

4.3.1.1.4 Dry matter content per grazing cycle

The response of dry matter (DM) content (%) to treatments was similar (P>0.05) in all the grazing cycles (Table 4.2). An increase in N application resulted in a decrease in DM content (Figure 4.8) when the treatments were averaged over the study period. Treatment Nvar and N0 had a higher (P≤0.05) DM content than treatments N60 and N80 but similar (P>0.05) to N20 and N40.

When DM content was averaged over treatments, grazing cycle data showed that from July to August in both 2016 and 2017, there was a decrease (P≤0.05) in DM content (Figure 4.9). From August 2016 to January 2017 the DM content of the pasture increased (P≤0.05) from 11.95% to 19.65%, similarly in August 2017 to January 2018 the DM content increased (P≤0.05) from 14.50% to 20.82%. The variation in DM content through both the grazing cycle and between year one and two might be due to differences in climatic conditions and botanical composition.

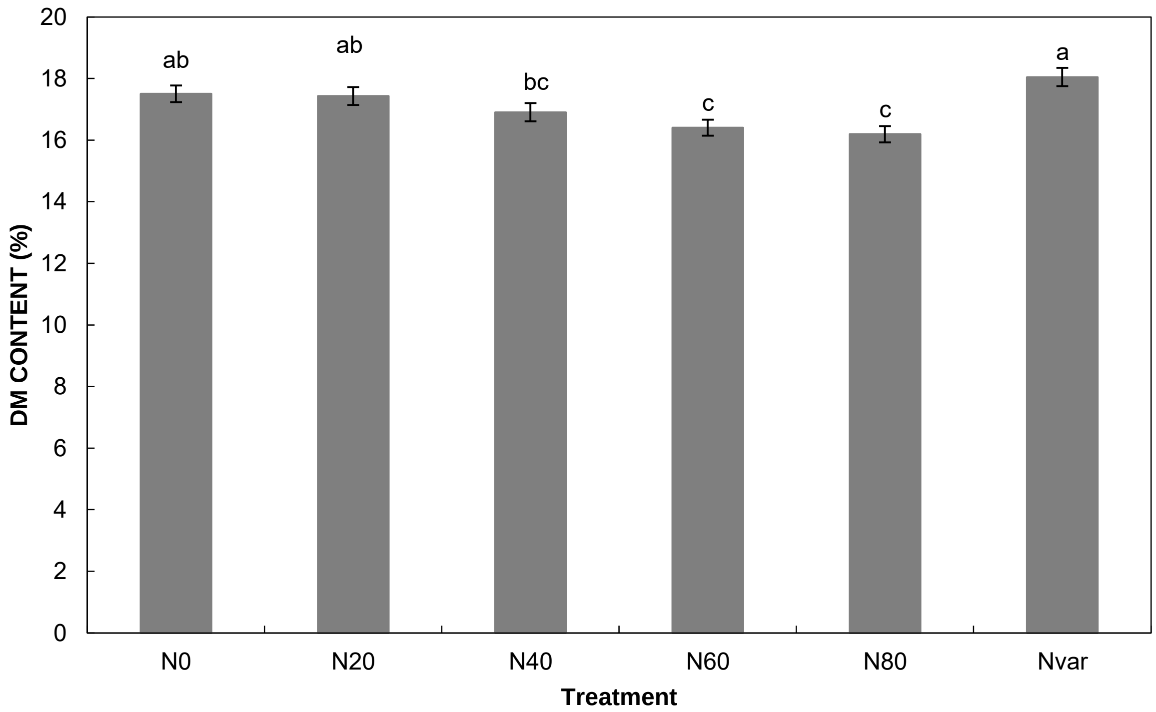

Figure 4.8: Average dry matter (DM) content (%) of the kikuyu site, when averaged over grazing cycles from May 2016 to March 2018 and affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$ and Nvar = varying N rate according to nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

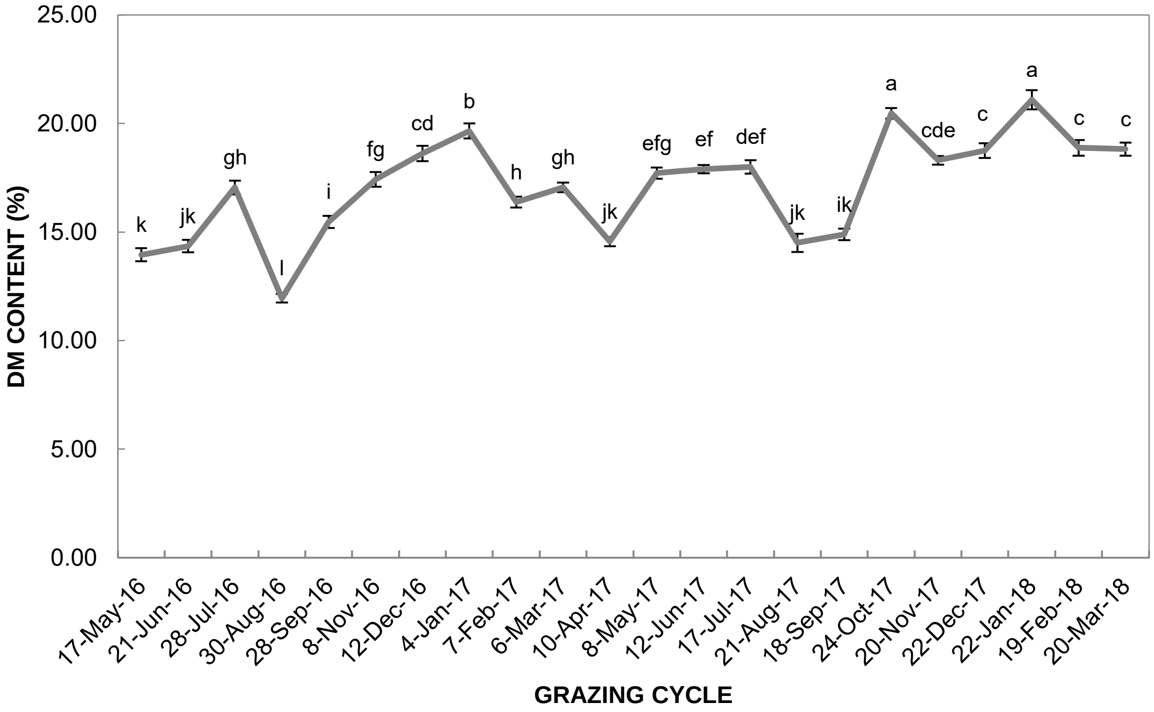

Figure 4.9: Dry matter (DM) content (%) of the kikuyu site, when averaged over treatments and affected by the grazing cycles. Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level.

4.3.1.1.5 Dry matter content per season

There was no interaction between treatment and season (P>0.05) that affected DM content, and thus the main effects of both treatment and grazing cycle are interpreted (P≤0.05) (Table 4.2). Treatment Nvar had a higher (P≤0.05) DM content than N80 in all the seasons, except autumn of year one and winter of year two (P>0.05) (Figure 4.10). Dry matter content in the winter of year one was generally lower (P≤0.05) for all treatments when compared to the other seasons (Figure 4.10). Of interest was that the dry matter content during winter of year one was generally lower compared to the treatments during winter in year two (P≤0.05) ,with the exception of treatment N0 and N60 being similar in winter of year one and in winter of year two (P>0.05). Summer of year two also had a higher (P≤0.05) DM content compared to treatments during summer of year one, except treatment N80 which remained similar (P>0.05). This could potentially indicate a build-up of fibrous material with a higher DM content than green vegetative material.

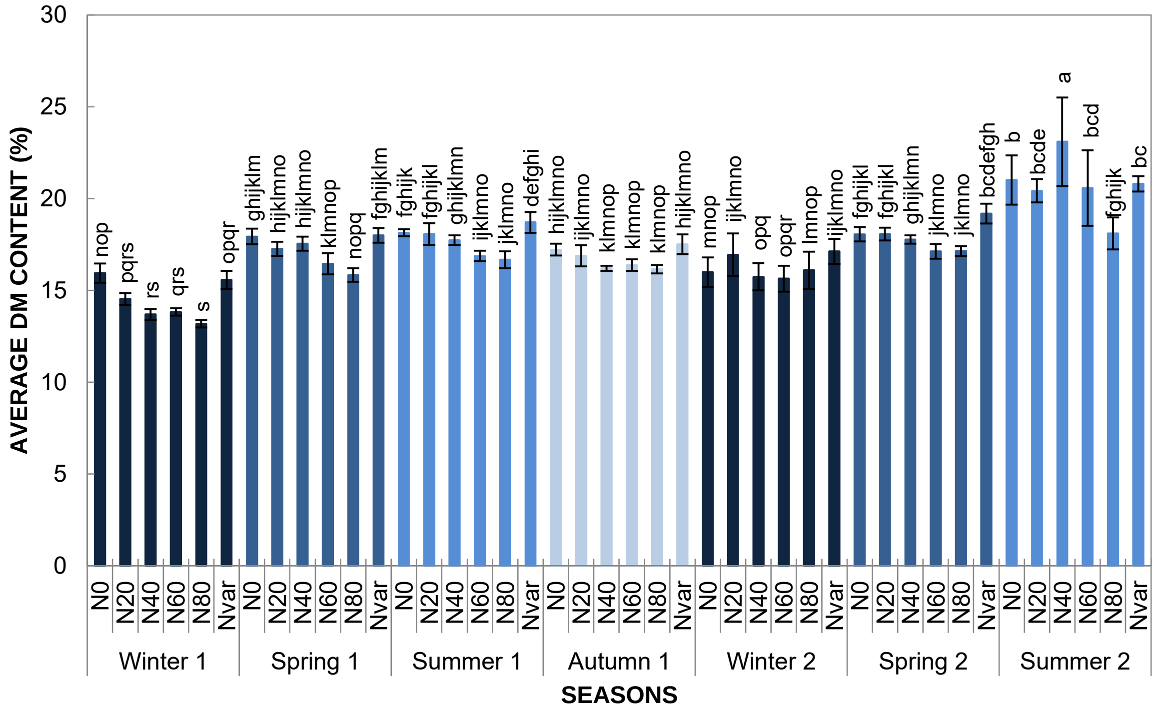

Figure 4.10: Average seasonal dry matter (DM) content (%) of the kikuyu site in seasons during year one (1) and two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60 and 80 kg N ha^{-1} and Nvar = varying rate of N. N Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

Kenney et al*.* (1984) reported a DM content of kikuyu in the vegetative state at about 15% in a sheep feeding experiment, but gave no indication of the rate of N fertilisation. In another sheep nutrition trial, kikuyu DM content were reported as 13.1% (Marais et al. 1990). Other authors have also reported that with low N treatments, higher DM contents can be expected (King et al. 2012; Bester 2014). Kikuyu pasture has also been reported to have a higher DM content compared to kikuyu over-sown with ryegrass pasture (Swanepoel et al. 2014; Botha 2003).

4.3.1.2 Botanical composition

4.3.1.2.1 Kikuyu

The response of kikuyu's contribution to the pasture varied (P≤0.05) across seasons and treatments (Table 4.2). From Figure 4.11, it is clear that kikuyu was dominant (P≤0.05) in the pasture during summer and autumn during both years of the study (Figure 4.11). Kikuyu made a low (P≤0.05) contribution to the pasture during spring of year one and two, and during winter of year two compared to the other seasons. Treatments did not have an effect (P>0.05) on the kikuyu component, during spring of both years. During winter of year one, N40 had the lowest (P≤0.05) contribution compared to all other treatments in the same season, while in winter of the second year, Nvar had a higher (P≤0.05) kikuyu content than N0 and N40. The non-conforming pattern seen as a result of treatments during winter of year one, might be due to carry-over effects from previous pasture management on the site. For instance, the site was previously over-sown with ryegrass and it might be that during winter, which is the part of the growing season for ryegrass, there was a degree of emergence and production from ryegrass allowed to set seed during previous years. It seems that N40 is the optimal amount for ryegrass growth during winter and the reason that kikuyu contributed lowest or similar to the lowest during winter of both years at the N40 treatment. The lower contribution of kikuyu during the second winter compared to the first, might be a reason that the DM production is also lower during the second winter compared to the first (Figure 4.4 and Figure 4.11).

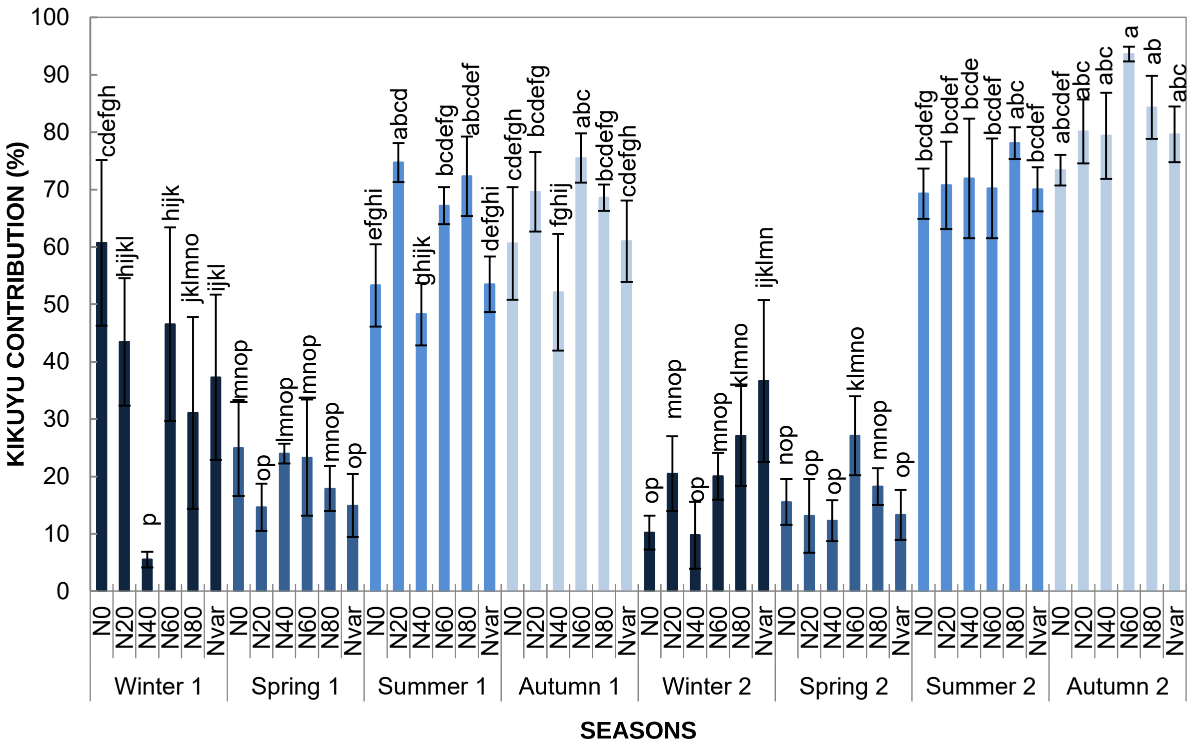

Figure 4.11: Kikuyu contribution (%) to the kikuyu site botanical composition in seasons during year one (1) and year two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$ and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

Overall it was also seen in New Zealand, that the kikuyu component was not affected by N treatment during August (spring) (Goold 1979). The current results are comparable with those found in Swanepoel et al. (2014) that the contribution of kikuyu was lower ($P \le 0.05$) during winter and spring than during summer and autumn. Similar results were found by van der Colf et al. (2015a) who reported an increase in the contribution of kikuyu from spring to autumn, while the contribution was similar during winter and spring under a kikuyu-ryegrass pasture. These results are representative of the growth period of kikuyu in warmer months (Harris and Bartholomew 1991; Marais 2001), while it approaches dormancy in winter and spring (Ward et al. 2013). As a result, kikuyu contributes more to the pasture, in terms of botanical composition, during summer and autumn.

Regarding the kikuyu component, summer and autumn of year one were affected (P≤0.05) by treatments however, summer and autumn of year two were not affected by treatments (P>0.05) (Figure 4.11 and Table 4.2). The reason for this is likely attributable to management prior to the start of the current study, which is that the site was over-sown with ryegrass. Volunteer ryegrass and its contribution therefore was potentially influenced by the N treatments to a greater degree than kikuyu on this particular site.

4.3.1.2.2 Volunteer ryegrass

For the volunteer ryegrass component there was no interaction (P>0.05) between treatment and season, but it was affected by both season and treatment as a main effects (P≤0.05) (Table 4.2). Ryegrass was not planted at the kikuyu site and was therefore termed as “volunteer ryegrass”.

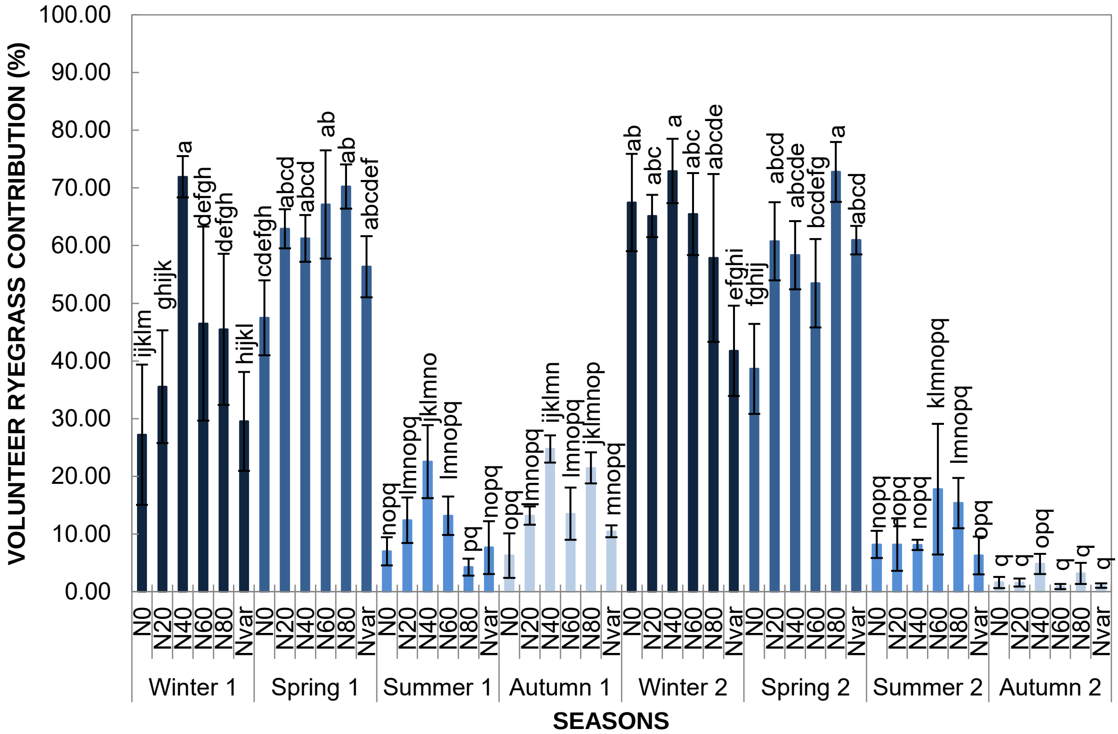

Figure 4.12: Volunteer ryegrass contribution (%) to the kikuyu site botanical composition in seasons during year one (1) and year two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$ and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

The contribution of volunteer ryegrass was affected by treatment (P≤0.05) in all seasons during both years of the study with the exception of summer and autumn of the second year (Figure 4.12). During summer and autumn of the second year, the contribution of kikuyu was overwhelming and out-competed the volunteer ryegrass. During this period (summer and autumn of the second year), the kikuyu component contributed between 69 to 94% compared to the volunteer ryegrass component which contributed 1 to 18%. Ryegrass is able to self-seed (Barth Neto et al. 2014) and that explains the possibility that ryegrass was the result from over-sowing it into the current site in years prior to the start of this study. The volunteer ryegrass contribution did not increase with the increase of N application rate as found by Nevens and Rehuel (2003) for ryegrass. This might be due to the fact that the volunteer ryegrass was not sown, resulting in poor establishment (patches) and as such obscuring the effects of N on its relative contribution.

In all seasons, although not always statistically significant ($P>0.05$), treatment N40 contributed the highest or similar to the highest amount of volunteer ryegrass (Figure 4.12). Kikuyu and volunteer ryegrass were negatively correlated, since as kikuyu contribution increased, the volunteer ryegrass amount decreased, and *vice versa* (Figure 4.14). The same trend was found by Swanepoel et al. (2014). In other studies on mixed swards, ryegrass content increased and clover content decreased with increasing amount of N fertiliser (Nevens and Rehuel 2003; Bolland and Guthridge 2007).

4.3.1.2.3 Volunteer legumes

The response of volunteer legumes was not the same ($P\leq0.05$) across treatments and seasons (Table 4.2). The volunteer legumes consisted mostly of white clover (*Trifolium repens*). In all seasons, except winter of year one, treatment N0 had the highest ($P\leq0.05$) or similar to the highest ($P>0.05$) contribution of volunteer legumes to the pasture compared to N60 (Figure 4.13). During the aforementioned seasons, volunteer legumes in treatment N0 contributed between 16 and 43% to botanical composition. For clover to make a positive contribution in terms of biological N fixation, a contribution of at least 30% is needed in the pasture (Clark and Harris 1996).

In treatment N0, the volunteer legume content increased ($P\leq0.05$) from winter to spring in both years and from spring to summer ($P\leq0.05$). It is advantageous to have clovers in the pasture because it has a NDF below 50% (Botha et al. 2008a), which promotes DM intake (Clark and Harris 1996). Summer had a higher ($P\leq0.05$) legume contribution compared to winter in the N0 treatment. Treatments N40, N60 and N80 had similar ($P>0.05$) contribution across all seasons except during winter of year one. This might be because the clover had not yet responded to the treatments in the first winter, since it is not its primary growth period. Harris et al. (1996) found that during late summer, clover content was at a maximum and during spring at a minimum. This was seen in the first year of the study but to a lesser extent during the second year, where the volunteer legumes contributed less during summer compared to spring ($P\leq0.05$). Numerous authors have reported on the decreasing clover component with an increase in N fertiliser application (Goold 1979; Elliott and Abbott 2003; Bolan et al. 2004).

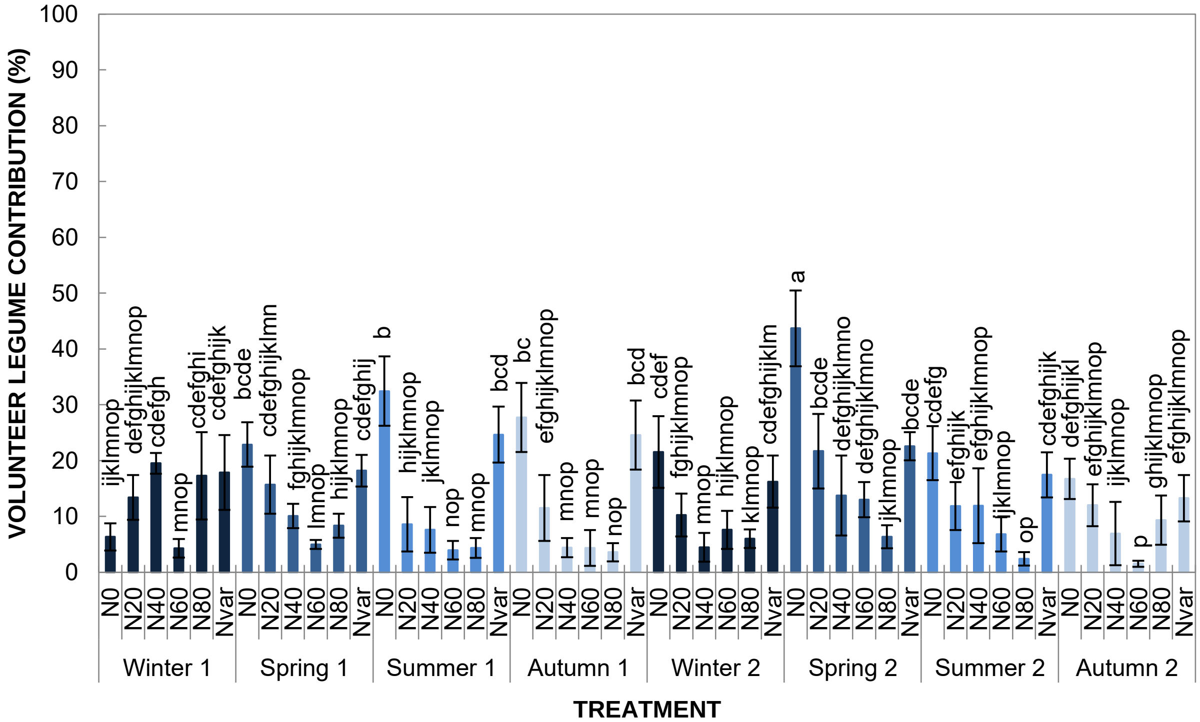

Figure 4.13: Volunteer legume contribution (%) to the kikuyu site botanical composition in seasons during year one (1) and year two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$ and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

4.3.1.2.4 Non-metric multidimensional scaling

The effect of N fertiliser rate on the botanical composition over seasons can be explained by a non-metric multidimensional scaling (NMDS) ordination analysis as indicated in Figure 4.14. Stress is intended to be lower than 0.2 and in this particular case, a two dimensional ordination resulted in stress of 1.8. When the distance of data points are far apart, regardless of their position, they differ most. When two arrows are pointing in opposite directions, a negative correlation exists between the two and the longer the arrow, the stronger the correlation. In Figure 4.14, N application is represented by the blue arrow and indicates the correlation between the amount of nitrogen applied and the ordination, while the botanical composition components are represented by the green arrows. The different shapes represent the different seasons while treatments are represented by different colours.

The correlation between the amount of N applied and the ordination was not significant (P=0.282) and R^2=0.01. The R^2 indicates the correlation coefficient between the ordination and the amount of N applied.

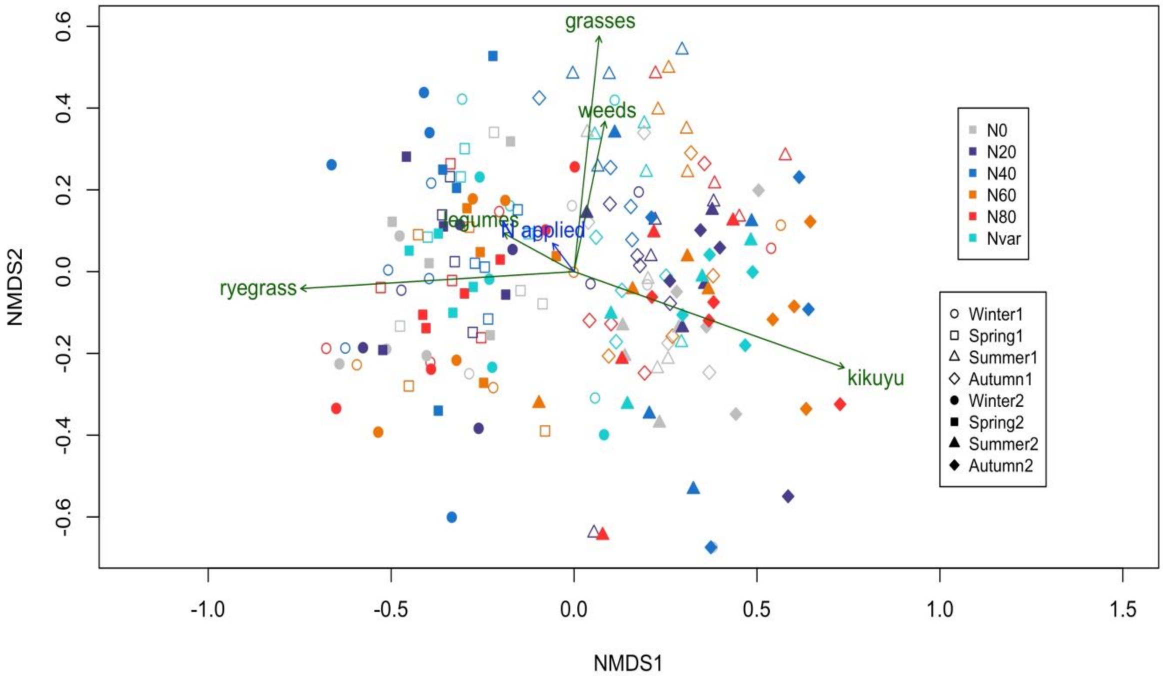

Figure 4.14: Nonmetric multidimensional scaling (NMDS) ordination, axis 1 and 2 of botanical composition components (kikuyu, volunteer ryegrass, volunteer legumes, other grasses and weeds) in the kikuyu site as influenced by season (winter, spring, summer and autumn of year one and two) and treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} and Nvar = varying N rate according nitrate concentration in the soil water

In Figure 4.14, axis one displays the change in botanical composition according to season, from kikuyu in summer and autumn to volunteer ryegrass in winter and spring. Axis two represents how many other grasses (besides kikuyu and volunteer ryegrass) and weeds were in the plots. Since there was no correlation between nitrogen and the ordinations, these differences are not the result of N application, but rather seasonal differences. It is also evident that both grasses and legumes were mostly present in the summer of the first year.

It might seem as if there is an increase in legumes with an increase in N application, but the correlation is weak (both arrows are short) and as mentioned earlier, there is no correlation between N application and the ordination. Therefore this is more likely to indicate a small increase from winter to spring in the second year. This is substantiated by Figure 4.13, where an increase in volunteer legumes is seen in the second spring.

4.3.1.3 Agronomic N use efficiency

The response of agronomic N use efficiency (ANUE) was similar across treatment and season (P>0.05) (Table 4.2). The treatments and season as main effects also had no effect (P>0.05) on the ANUE.

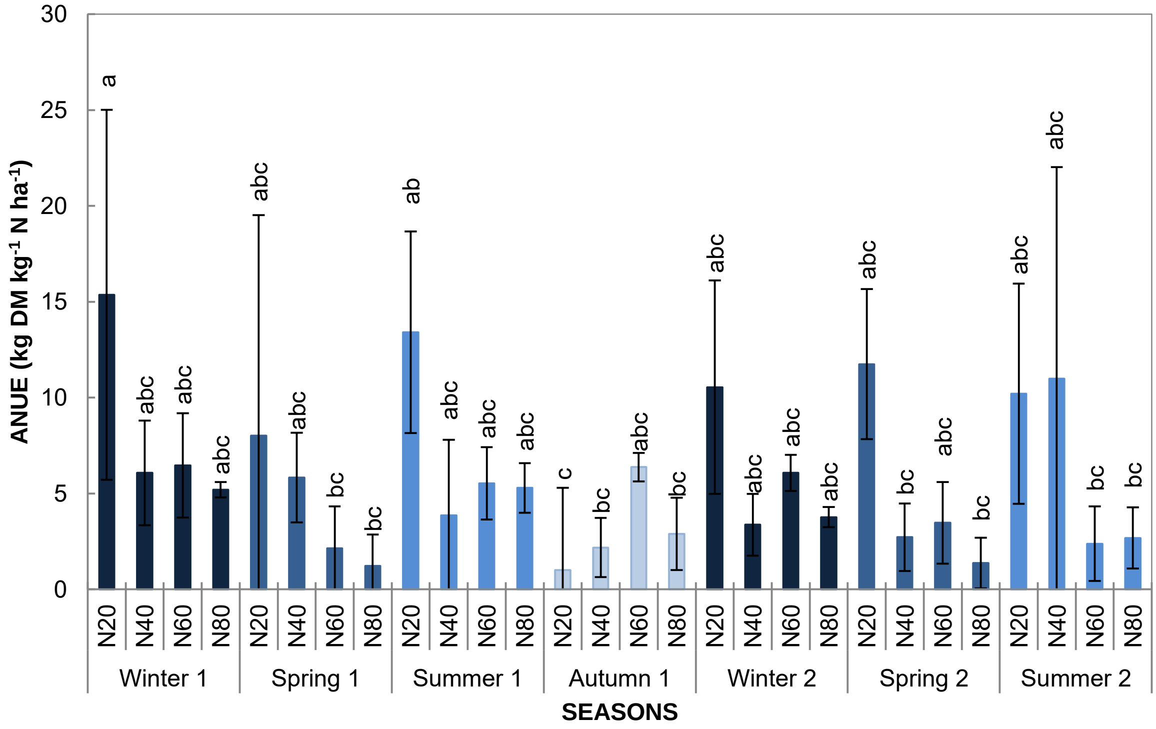

Figure 4.15: Agronomic nitrogen use efficiency (ANUE) (kg DM kg^{-1} N ha^{-1}) of the kikuyu site in seasons of year one (1) and two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar = varying N rate according nitrate concentration in the soil water.. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

Agronomic NUE were variable in its responses to treatments on a grazing cycle basis (data not shown). This is similar to what is found by Bolland and Guthridge (2007), which is that efficiency varies between years and experiments. Cook and Mulder (1984) found that during high production periods between ANUE values of between 17.8 and 28.8 kg DM kg^{-1} N ha^{-1} can be expected, with other authors confirming this with increases of up to 30 kg DM per kg^{-1} N ha^{-1} reported (Tamimi et al. 1968; Miles 1997). These production responses were also seen, regardless of N treatment, when looking at monthly ANUE. During summer months of the first and second year, a maximum ANUE value of 33 and 31 kg DM kg^{-1} N ha^{-1} was obtained. Previous studies have found that when kikuyu experienced low temperatures and moisture stress, N often did not have an effect on pasture growth (Goold 1979). A reason that treatment differences are not observed in the current trial may be due to the existing N reserves in the soil and recycling through animals (Cross 1979), resulting in all treatments having access to more N than what is applied through inorganic N fertiliser. Figure 4.15, although not indicating significant differences (P>0.05) and variation across seasons, can be used to build on the idea of strategic fertilisation based on season.

4.3.1.4 Crude protein

Crude protein (CP) content was not affected by grazing cycle and treatments in a similar manner (P≤0.05) (Table 4.2). There was a general trend for lower N application resulting in lower CP of pasture. Pearson et al. (1985) also reported similar trends of N rate affecting the CP content in kikuyu. In South Africa the CP content of kikuyu was found to be in the range of 16.9 and 22.5% (Marais et al. 1990) while in the Eastern Cape specifically, CP is in the range of 8.31 to 33.3%, depending on the amount of N applied (200 – 600 kg N ha^{-1} y^{-1}) (Miles et al. 2000). On the current study site, other authors such as Botha (2003) and Botha et al. (2008b) found CP content in the range of 21.1 to 23.7% when fertilised at a rate of 60 kg N ha^{-1} grazing $cycle^{-1}$. This is similar to what is found for the same treatment (N60) in the current study. The CP content in the current study ranged from a minimum of 12.8% (N20 in November 2016) to a maximum of 30.8% (N80 in August 2016) (monthly CP data not shown). The CP content requirement for a small breed lactating dairy cow is 15% (NRC 2001), which means that 30.8% is well in excess of this requirement. During spring and summer grazing cycles, treatments N0, N20 and Nvar were mostly within an acceptable range regarding the requirement of the grazing animals. Fertilisation to optimise forage quality of the pasture thus seems to be within the range of these treatments. Kikuyu has been reported to have a lower non-protein N content compared to perennial ryegrass (221 compared to 245 g kg^{-1} CP, respectively) (Reeves et al. 1996). Higher non-protein N results in lower DM intake of kikuyu pasture (Dugmore and du Toit 1988). This is similar to what Fulkerson et al. (2007) found during autumn. The author however found that kikuyu had a higher non-protein N content during summer compared to perennial ryegrass. A similar seasonal pattern of non-protein N, in other words higher in summer and lower in autumn, was found by Dugmore and du Toit (1988). The high values for CP seen in some treatments during the study may thus have negative effect on animal health if there is a large NPN component.

Seasonal CP content differed ($P \leq 0.05$) in response to treatment and season (Figure 4.16). The kikuyu pasture had the highest average seasonal CP content during winter and autumn, and the lowest during spring and summer. Similar seasonal trends were found by Fourie (2015) and Meeske et al. (2006). In the current study, during winter and autumn, the CP content found in treatment N0 and Nvar was lower ($P \leq 0.05$) compared to N40, N60 and N80. In spring, N0, N20, N40 and Nvar were equally low ($P > 0.05$) in CP content. During spring, treatments N0, N20, N40 and Nvar were lower ($P \leq 0.05$) compared to N60 and N80. Treatments N0, N20 and Nvar were the lowest ($P \leq 0.05$) during summer, compared to N60 and N80, but with N20 being similar ($P > 0.05$) to N40. Various authors reported that the lowest CP was during spring for kikuyu (Pearson et al. 1985; Fulkerson et al. 1999). Meeske et al. (2006) found, on a similar location than the current study, that CP on the 21.8% (winter), 17.6% (spring), 15.7% (summer) and 19.6% (autumn). This is within similar ranges to the current study.

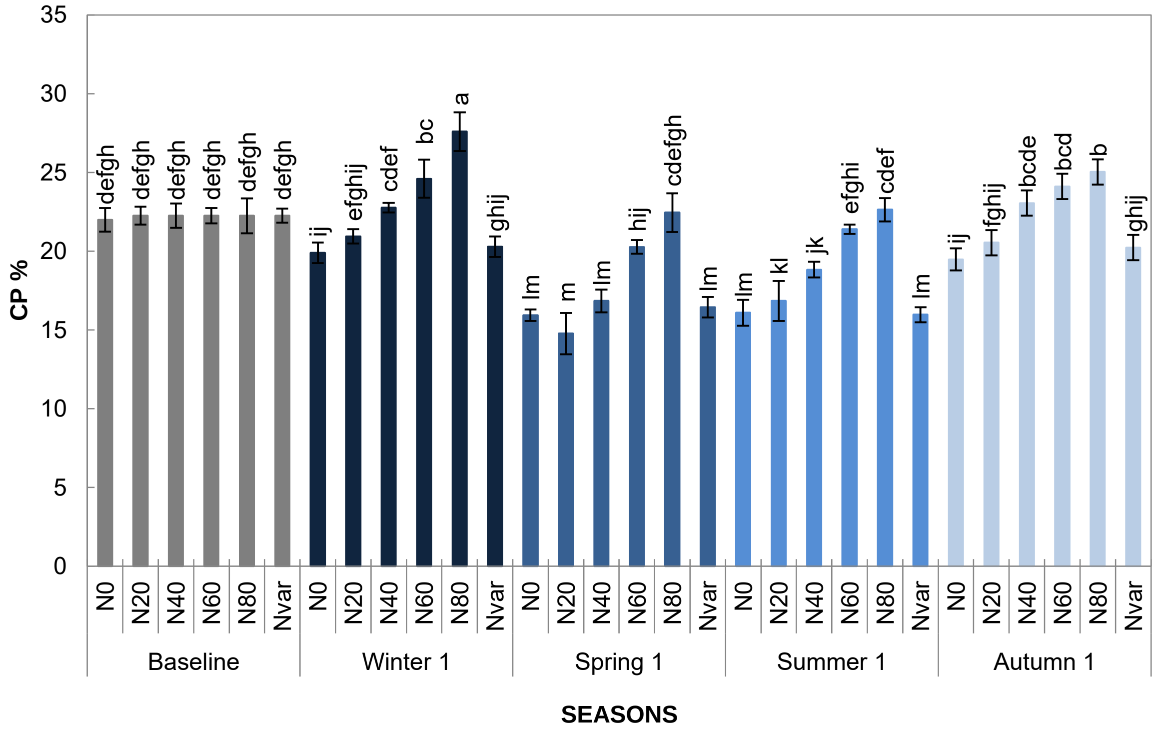

Figure 4.16: Average crude protein (CP) content (%) of the kikuyu site in seasons of year one (1) and affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

4.3.2 Kikuyu-ryegrass pasture site

4.3.2.1.1 Herbage production per grazing cycle

The response of herbage production to treatments were similar (P>0.05) in all grazing cycles (Table 4.4). When the DM production per grazing cycle was averaged over the study period (May 2016 to March 2018), N0 produced less (P≤0.05) herbage than N60 and N80, and had a similar (P>0.05) herbage production compared to Nvar, N20 and N40 (Figure 4.17). When averaged over treatments, herbage production decreased (P≤0.05) from May to July in 2016 and from May to July in 2017 (Figure 4.18). Grazing capacities of kikuyu over-sown with Italian ryegrass were reported by van der Colf et al. (2015b) on the same site to be the lowest during July. During the current study the highest (P≤0.05) production was recorded in November during year 1. Similar to what was found on the kikuyu site, this was due to a long grazing cycle. This was eliminated by calculating the growth rate (Figure 4.19). The production per grazing cycle during the second year (June 2017 – March 2018) was much lower compared to the grazing cycles in the first year (June 2016 – May 2017). The growth rate was not the same over the two years of the study period and this is likely attributed to climatic influences (Figure 4.19).

4.3.2.1.2 Seasonal herbage production

The response of cumulative seasonal herbage production to treatments did not vary (P>0.05) between seasons (Table 4.4). Treatment and season, as main effects, affected (P≤0.05) the herbage production (Figure 4.20). Within the spring and summer season of year one, production for all treatments, except N60, were similar (P>0.05). The production of spring and summer herbage of year one was higher (P≤0.05) compared to winter and autumn of year one, with the exception of N40. Treatment N40 had similar (P>0.05) production during winter and summer of year one. No treatment differences (P>0.05) were found in winter and spring of year two and during autumn of year one. During spring of year one, N60 produced a higher (P≤0.05) cumulative herbage compared to all other treatments except N80 (P>0.05). During summer of year one, N60 produced (P≤0.05) higher than N40 but similar (P>0.05) to all other treatments. Summer of year two resulted in treatment N80 having a higher (P≤0.05) herbage production compared to N0, but the production during other seasons was similar (P>0.05) for all treatments.

In a study in the USA, annual ryegrass produced between 5.82 to 11.58 t DM ha^{-1} during year one and between 4.40 to 10.66 t DM ha^{-1} during the growing season (first harvest was during winter in December) of year two when N was applied at 280 kg N ha^{-1} (Venuto et al. 2004). They attributed the differences to location, year, cultivar and seeding rate.

From May until November, yields of 5.9, 10.0, 13.0, 13.8 and 13.1 t DM ha^{-1} were obtained at rates of 0, 20, 40, 60 kg N ha^{-1} and according to nitrate concentration of the soil, respectively, for an annual ryegrass pasture in a study conducted by (Fessehazion et al. 2011) on a pure ryegrass stand on Cedara, South Africa. The results of the current study for similar N rates and time period (May to November) were 10.8, 12.8, 12.6, 13.6 and 12.3 t DM ha^{-1}. Thus all treatments had a higher yield than what Fessehazion et al. (2011) recorded, except in the N60 and Nvar treatments. The reason might be that there is high residual N in the soil of the current study when comparing the N0 (5.9 vs 10.8 t DM ha^{-1}) treatment in particular. The residual effect was also observed by King et al. (2012) when fertilising with N increased the DM production, but resulted in a lower than expected effect due to high residual N in the soil. As described in Chapter 3 , the use of the WFD to obtain nitrate concentration to determine the Nvar was not as successful as in Fessehazion et al. (2011). The authors reported that a compromise between quality and quantity of herbage production was achieved at a nitrate concentration of 50 mg L^{-1} and therefore fertilised according to that critical concentration. In this study however, this was not the case, as the Nvar achieved similar yields to the N0 treatment. As a result, the recommendation was adapted as described in Chapter 4 (section 4.2.2.1). Reasons why the current study's critical nitrate concentration differed to that of Fessehazion et al. (2011) is not clear. It might be possible that the soil type had an influence, since Fessehazion et al. (2011) conducted the study on a deep (approximately 1 m), red, kaolinitic Hutton soil, compared to a more shallow podzol soil, specifically characterised as a

Witfontein soil form in the current study. It might result in the roots being able to take up water and nutrients from deeper levels as ryegrass roots are able to grow the depth of 1m (Høgh-Jensen and Schjoerring 1997).

On the same location as the current study, seasonal production of 1.72, 5.96, 7.38, 5.26 t DM ha^{-1} for winter, spring, summer and autumn, respectively, was found (Swanepoel et al. 2014). Winter production was lower compared to what was recorded on the current study during winter for the first year (5.3 t DM ha^{-1}), while winter of the second year (2.5 t DM ha^{-1}) was more comparable to what Swanepoel et al. (2014) found. This is due to one less grazing cycle that contributed to the second winter season compared to the first. Spring production (7.26 t DM ha^{-1}) was higher and summer production (6.62 t DM ha^{-1}) was lower in the study by Swanepoel et al. (2014) compared to the current study. Autumn production was higher (5.26 t DM ha^{-1}) than that of the current study (3.68 t DM ha^{-1}). During the second year, spring and summer production on the current study (5.15 t DM ha^{-1} and 5.60 t DM ha^{-1} respectively) was lower.

The herbage production estimated by pre-grazing disc meter readings is shown in Table 4.5 and the response varied ($P \leq 0.05$) between treatment and season (Table 4.4). The regression equations used to calculate the estimated herbage production was developed for Italian ryegrass sown into kikuyu (van der Colf 2011). The seasonal production was found to be 3.51, 6.07, 6.16 and 3.02 t DM ha^{-1} during winter, spring, summer and autumn respectively for Italian ryegrass (cv. Jeanne) sown into kikuyu (van der Colf et al. 2015a). This is also the site were the regression equations was developed. The reason why the estimated production is lower than the herbage production determined through cutting samples in the current site, might be due to the regressions being developed on a lower producing ryegrass variety than the one in the current study (cv. Barmultra II). This is confirmed by ryegrass variety trials on the Outeniqua Research Farm where Barmultra outperformed Jeanne on annual production (van der Colf 2016).

Table 4.4: ANOVA of kikuyu-ryegrass site. Num DF=Numerator degrees of freedom, Den. DF= Denominator degrees of freedom

	Num. DF	Den. DF	F	P-value
Herbage production				
Treatment	5	18	3.07	0.018
Grazing cycle	20	360	39.61	<0.001
Treatment* Grazing cycle	100	360	0.82	0.877
Growth rate				
Treatment	5	18	4.03	0.013
Grazing cycle	19	342	91.11	<0.001
Treatment* Grazing cycle	95	342	1.29	0.055
Dry matter content				
Treatment	5	18	4.88	0.005
Grazing cycle	20	360	52.00	<0.001
Treatment* Grazing cycle	100	360	0.93	0.675
Herbage Production				
Treatment	5	18	3.47	0.023
Season	7	126	314.63	<0.001
Treatment* Season	35	126	1.02	0.455
Pre-grazing Disc meter				
Treatment	5	18	13.70	<0.001
Season	7	126	260.17	<0.001
Treatment* Season	35	126	3.31	<0.001
Growth rate				
Treatment	5	18	4.77	0.006
Season	7	126	148.74	<0.001
Treatment* Season	35	126	1.02	0.445
Post-grazing: Disc meter				
Treatment	5	18	0.27	0.925
Season	7	126	32.13	<0.001
Treatment* Season	35	126	0.71	0.882
Dry matter content				
Treatment	5	18	4.48	0.008
Season	7	126	48.10	<0.001
Treatment* Season	35	126	1.18	0.249
Botanical composition: Kikuyu				
Treatment	5	18	0.46	0.799
Season	7	126	109.50	<0.001
Treatment* Season	35	126	1.44	0.076
Botanical composition: Ryegrass				
Treatment	5	18	4.34	0.009
Season	7	126	175.57	<0.001
Treatment* Season	35	126	1.39	0.098
Botanical composition: Volunteer Legumes				
Treatment	5	18	11.82	<0.001
Season	7	126	17.10	<0.001
Treatment* Season	35	126	2.27	0.001
Agronomic N use efficiency				
Treatment	3	12	0.47	0.712
Season	7	84	4.79	<0.001
Treatment* Season	21	84	0.84	0.663
Crude Protein				
Treatment	5	18	34.95	<0.001
Season	5	89	88.50	<0.001
Treatment* Season	25	89	3.35	<0.001

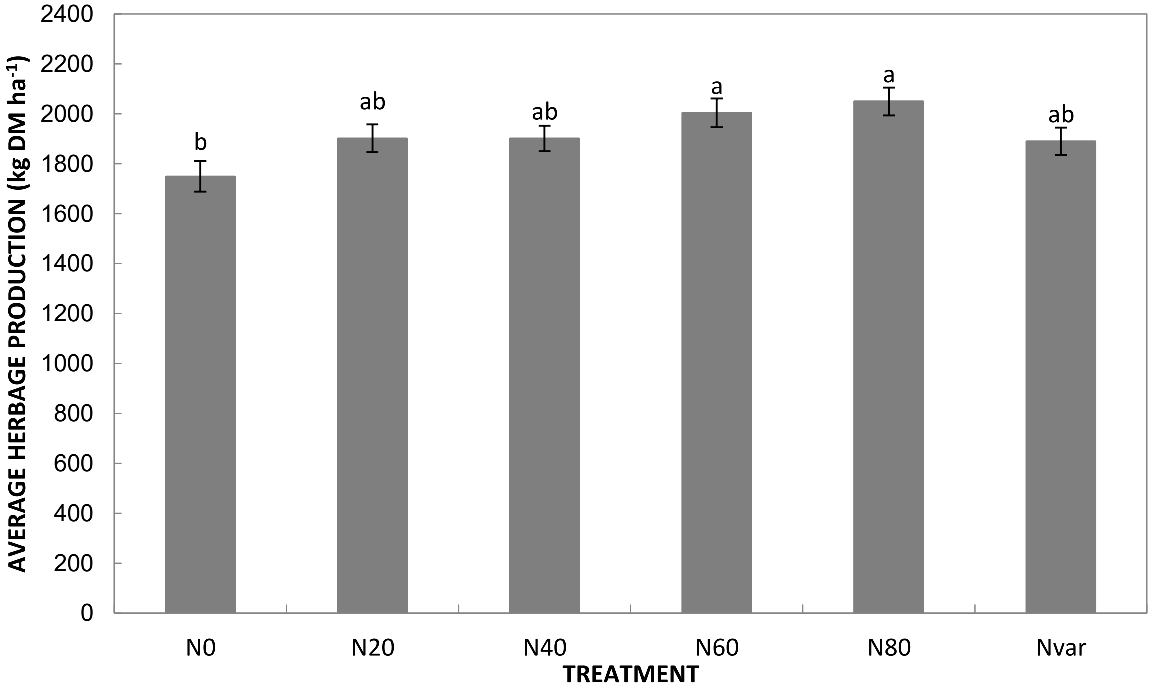

Figure 4.17: Average herbage production (kg DM ha^{-1}) of kikuyu-ryegrass site when averaged over grazing cycles from May 2016 to March 2018, as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar = varying N rate according to nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicate significant difference at a 5% level

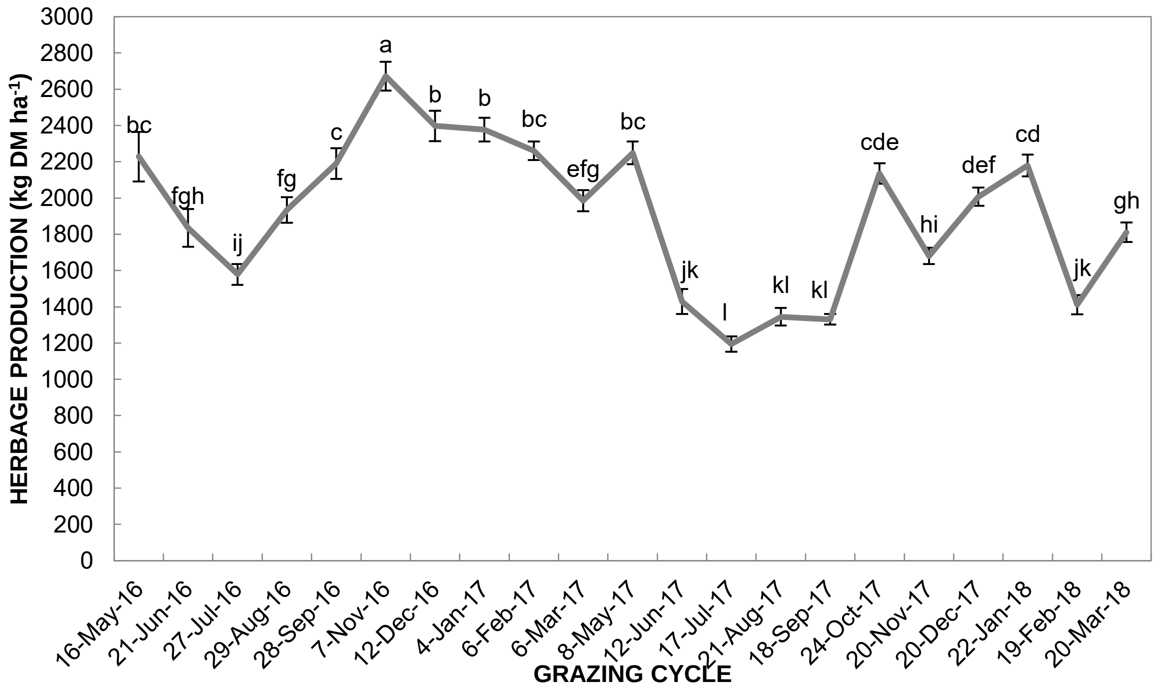

Figure 4.18: Average pasture herbage production (kg DM ha^{-1}) of the kikuyu-ryegrass site when averaged over treatments during the different grazing cycles of the study period. Error bars indicate standard error. No common letter above data points indicatesignificant difference at 5% level

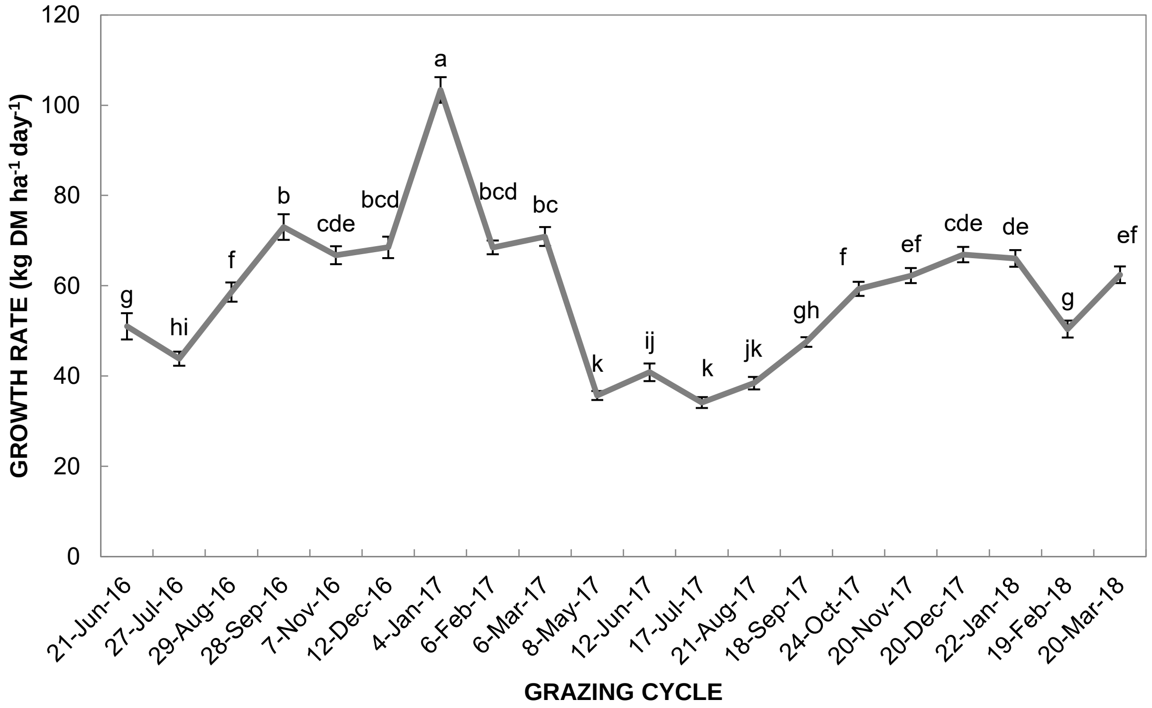

Figure 4.19: Mean pasture growth rate (kg DM ha^{-1} day^{-1}) of kikuyu-ryegrass site when averaged over treatments during the different grazing cycles of the study period. Error bars indicate standard error. No common letter above data points indicatesignificant difference at 5% level

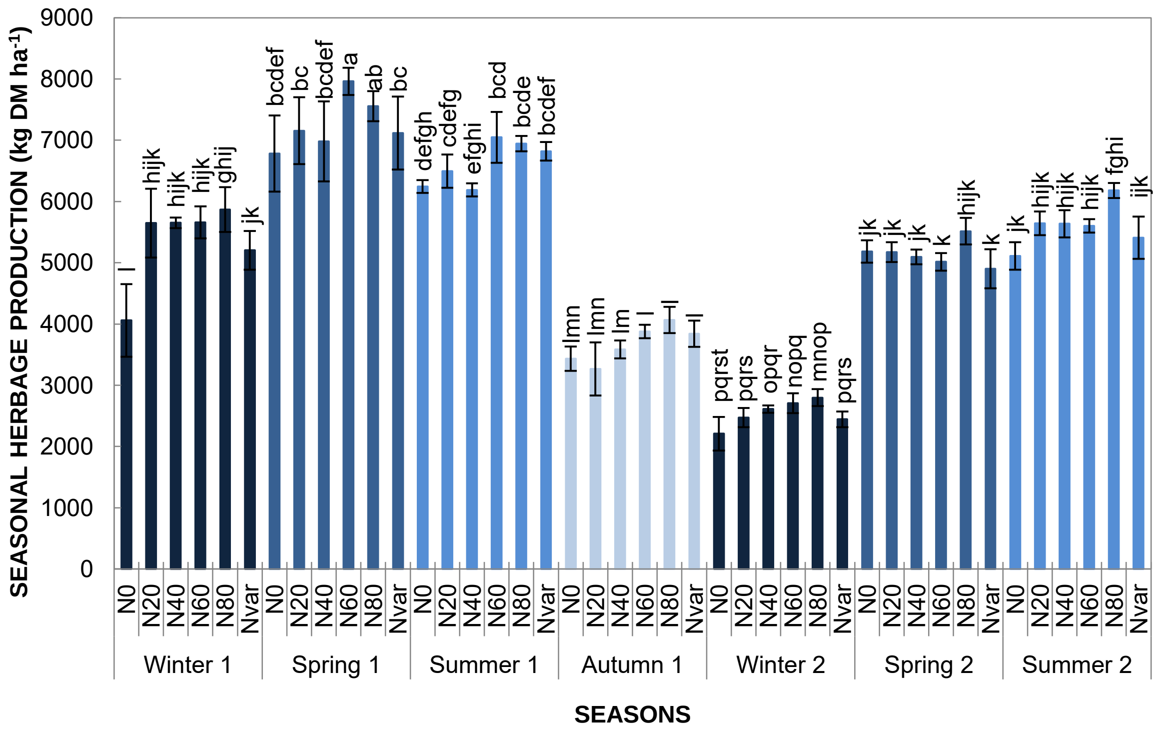

Figure 4.20: Seasonal herbage production (kg DM ha^{-1}) of the kikuyu-ryegrass site in seasons during year one (1) and two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar= varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

Table 4.5: Average seasonal herbage production (t DM ha^{-1}) of year one and two, estimated by pre-grazing disc meter readings on kikuyu-ryegrass pasture for treatments N0, N20, N40, N60, N80 and Nvar using regressions for kikuyu-italian ryegrass pastures (van der Colf 2011)

	Winter 1	Spring 1	Summer 1	Autumn 1	Winter 2	Spring 2	Summer 2
N0	2.33 qrstu	2.98 mnop	3.24 lmn	2.04 rstuvw	1.72 vwx	3.83 ijk	4.06 fghi
N20	3.38 klm	3.88 hijk	3.84 ijkl	2.22 qrstuvw	1.92 stuvwx	4.55 defg	4.83 cde
N40	4.01 fghij	4.47 defgh	3.80 ijkl	2.37 pqrstu	2.23 qrstuv	4.89 bcde	5.30 abc
N60	4.92 bcde	4.61 def	3.90 hijk	2.81 mnopq	2.36 pqrst	4.84 cde	5.05 abcd
N80	4.90 cde	5.32 abc	4.10 fghi	2.69 nopq	2.55 opqr	5.52 ab	5.63 a
Nvar	3.00 mno	3.39 jklm	3.86 hijk	2.30 qrstuv	1.88 stuvw	3.95 ghijk	4.35 efghi

N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} and Nvar = varying N rate according nitrate concentration in the soil water

On cutting trials in Queensland, Australia, the optimum N application rates to achieve 90% of the maximum yield was found to range between 52 to 112 kg N ha^{-1} $month^{-1}$ during the growing season of short term ryegrass (Lowe et al. 2005). These wide ranges are due to different sites and results from different years. Eckard (1989) suggested that N application rates for cutting trials be reduced with 25% when fertilising pastures that are grazed in order to adjust for the return of nutrients to soil. This will result in 39 to 84 kg N ha^{-1} $month^{-1}$ with grazing in the study of Lowe et al. (2005). In Tasmania, Australia, a strategic fertiliser application of no more than 60 kg N ha^{-1} is suggested on grazed perennial ryegrass-clover pasture, especially when temperatures are low, so that during the warmer months the positive effects of clover could still be utilised (Eckard and Franks 1998). In South Australia, guidelines for fertilising perennial ryegrass include four to six applications during the growing season at rates of 25 to 50 kg N ha^{-1} $application^{-1}$ (Elliott and Abbott 2003). In New Zealand optimum N rates were based on the N concentration in the herbage. When the N concentration was between 2.5 to 2.8% or between 3.7 to 4% the N application rate differed from 160 to 60 kg N ha^{-1} respectively to obtain similar yields (Vogeler and Cichota 2016). In South Africa, optimum rates in the Natal midlands were described as 300 to 350 kg N ha^{-1} $year^{-1}$ (Eckard 1989) which is much lower than that in Australia, justifying the need to develop area specific N requirements. Low carry over effects was seen in 30 to 60 kg N ha^{-1} compared to 120 and 180 kg N ha^{-1} in a pot trial on perennial ryegrass (Labuschagne and Agenbag 2006). The strategic N fertiliser through time on this kikuyu-ryegrass site is complicated by the fact that there were not similar responses to treatments in the same seasons during the two year study. Based on the results in this study it would seem that 20 kg N ha^{-1} $month^{-1}$ during winter, increasing to 60 kg N ha^{-1} $month^{-1}$ during spring and summer and decreasing as low as 0 kg N ha^{-1} $month^{-1}$ during autumn for the first year on kikuyu-ryegrass pasture, is viable. However, during winter and spring of the second year no N was needed to obtain similar production to fertilised treatment, while up to 80 kg N ha^{-1} month^{-1} during the second summer is needed for a production higher compared to that of no N.

The seasonal growth rate (Figure 4.21) also showed that the growth rates were not the same for seasons during year one compared to those in year two, making it difficult to construct a common strategic fertiliser regime according to season. These differences between seasons of the two years might be due to climatic differences such as temperature and rainfall.

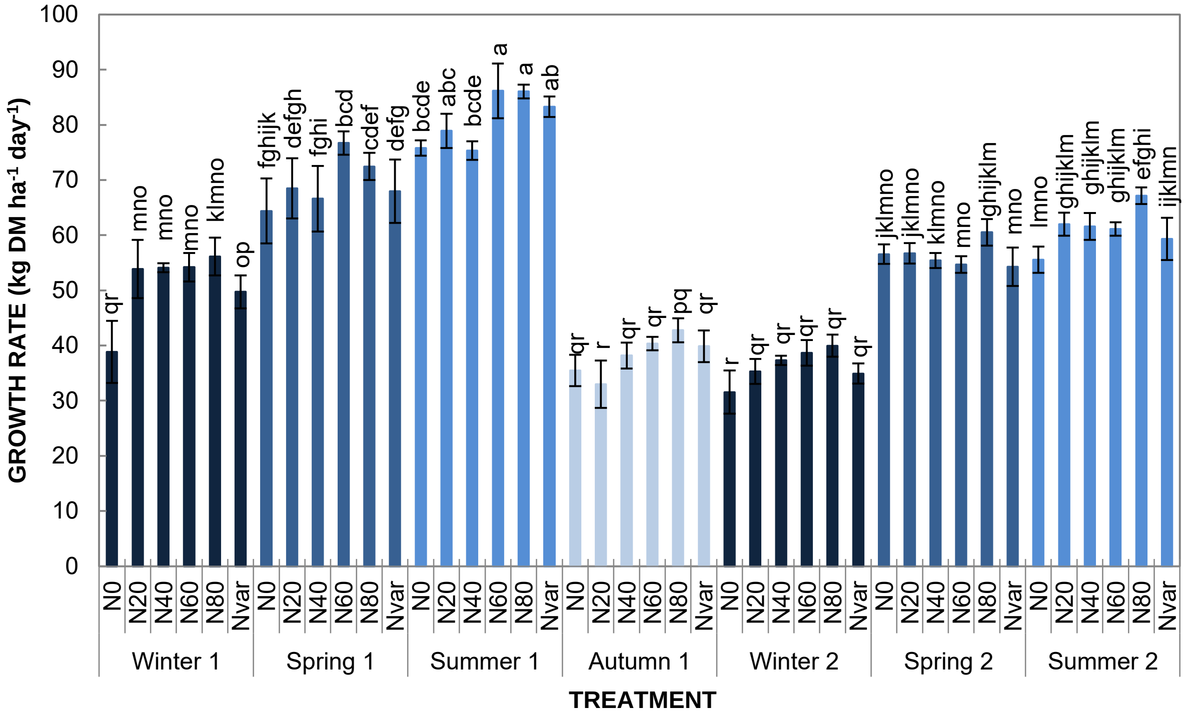

Figure 4.21: Average seasonal growth rate (kg DM ha^{-1} day^{-1}) of the kikuyu-ryegrass site in seasons during year one (1) and two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

4.3.2.1.3 Total annual herbage production

Annual herbage production for year one was influenced ($P \leq 0.05$) by the treatment. Treatment N60 and N80 had a higher ($P \leq 0.05$) herbage production than N0, but similar ($P > 0.05$) production to Nvar, N20 and N40 (Figure 4.22). Swanepoel et al. (2014) found an annual herbage production of 20.33 kg DM ha^{-1} for kikuyu-ryegrass receiving approximately 32 kg N ha^{-1} grazing^{-1}, in the similar location as the current study. This is comparable to the N0 treatment. Annual yields of 16, 15.7 and 19.3 kg DM ha^{-1} at 600 kg N ha^{-1} (applied in 10 split dressings) were found by Botha et al. (2008a), also lower to what was found in the current study. Total herbage production of Italian ryegrass sown into kikuyu was found to be 16.55 and 17.65 t DM ha^{-1} in the KwaZulu-Natal Province of South Africa receiving 50 kg N ha^{-1} after every harvest (Harris and Bartholomew 1991).

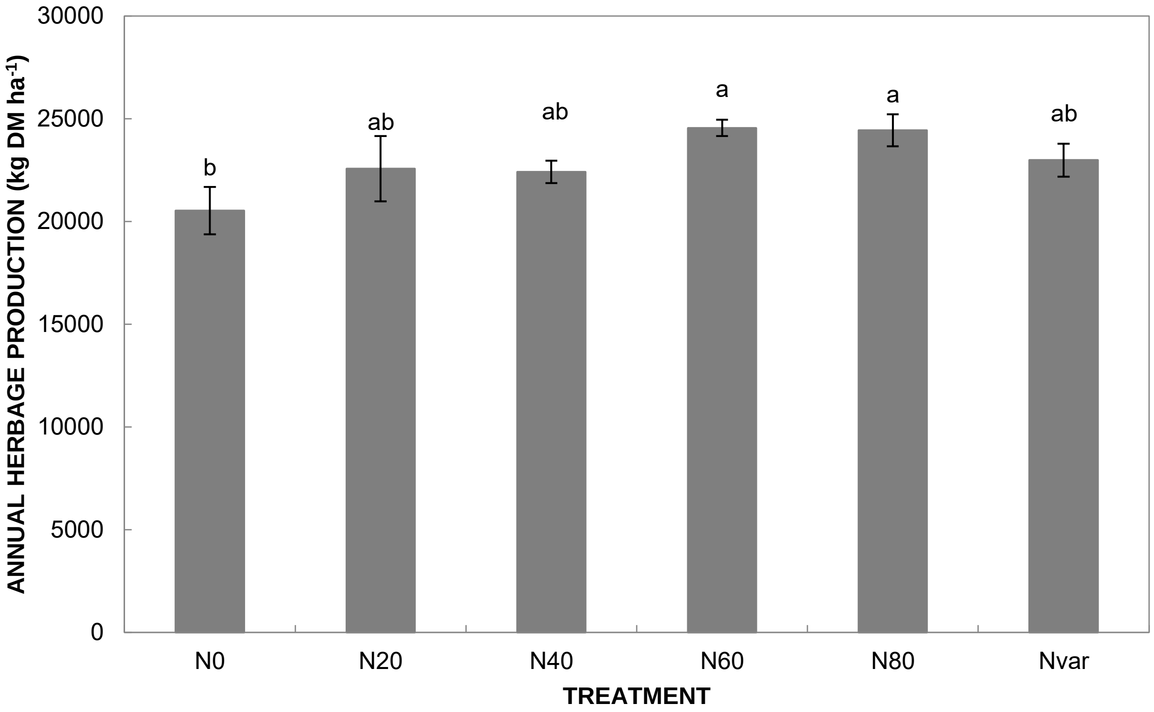

Figure 4.22: Annual herbage production (kg DM ha^{-1}) of the kikuyu-ryegrass site as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60 80 kg N ha^{-1} and Nvar = varying rate of N application according to soil water nitrate concentration. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

Post-grazing disc meter readings were taken in order to estimate the pasture remaining after grazing 30 mm above ground level (Table 4.4). The average amounts of material remaining after every grazing within the seasons ranged between 109 and 489 kg DM ha^{-1} and were mostly affected by season (Figure 4.23). Post-grazing regressions for Italian ryegrass sown into kikuyu (van der Colf 2011) were used. The kikuyu-ryegrass site had lower post grazing material compared to the kikuyu site and this might be due to the better quality of ryegrass in the pasture compared to mostly kikuyu in the kikuyu site. This could be confirmed by exploring the neutral detergent fibre (NDF) content. On the same farm it was found that during spring kikuyu had a higher NDF compared to kikuyu-ryegrass pastures which usually had a NDF value of 50% or lower. Both kikuyu and kikuyu-ryegrass had a NDF content higher than 60% during summer and autumn. It should be noted that with a NDF above 50% a negative effect on intake can be expected (Botha et al. 2008a).

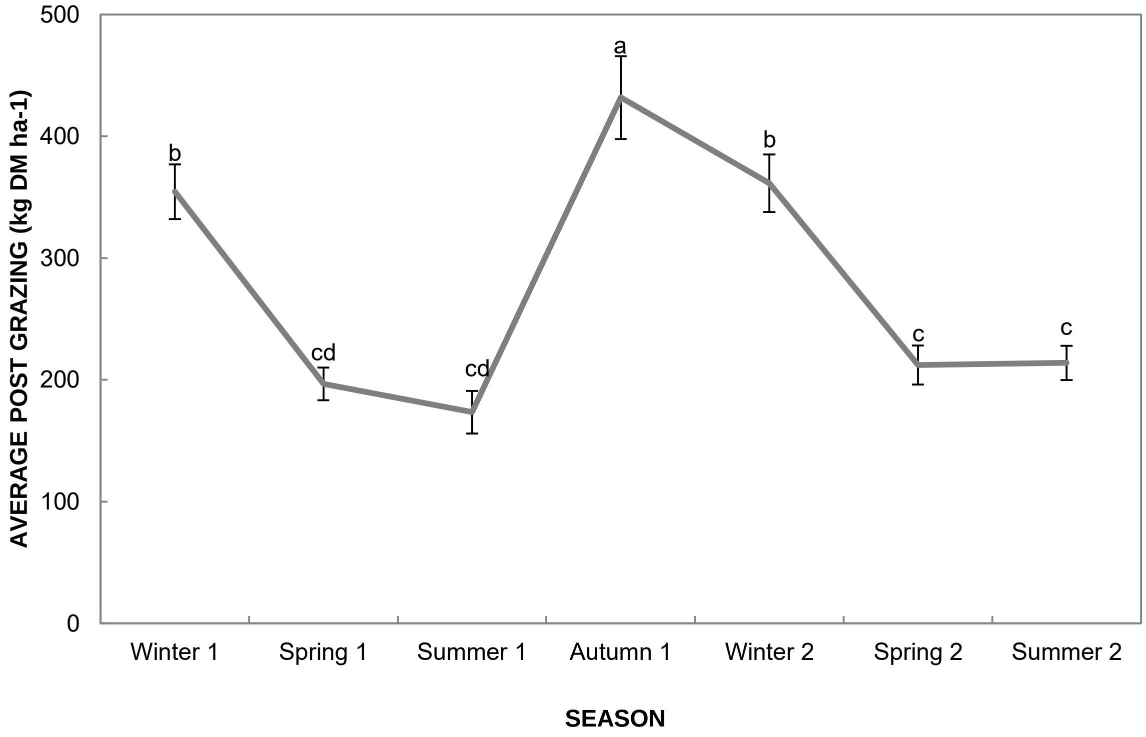

Figure 4.23: Average post-grazing (kg DM ha^{-1}) pasture yield of the kikuyu-ryegrass site as affected seasons during year one (1) and two (2). Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level

4.3.2.1.4 Dry matter content per grazing cycle

The response of dry matter (DM) content (%) to treatment were similar (P>0.05) in all grazing cycle (Table 4.4). When averaged over the study period (May 2016 to March 2018), DM content in treatments N0, N40 and Nvar was higher (P≤0.05) than in N60 and N80, but similar (P>0.05) to N20 (Figure 4.24). This is similar to what was found by Theron and van Rensburg (1998) on a pure ryegrass stand, where a decrease in N application resulted in an increased DM content. They also found that as the defoliation interval increased, the DM content also increased.

In Figure 4.25, the higher (P≤0.05) DM content in December 2016 and January 2017 and 2018 may be due to the ryegrass approaching the end of its growing season and the kikuyu starting to grow, creating more fibrous material. Kikuyu has a higher overall DM content than ryegrass (Swanepoel et al. 2014), and would thus increase the DM content as its content increases in the pasture.

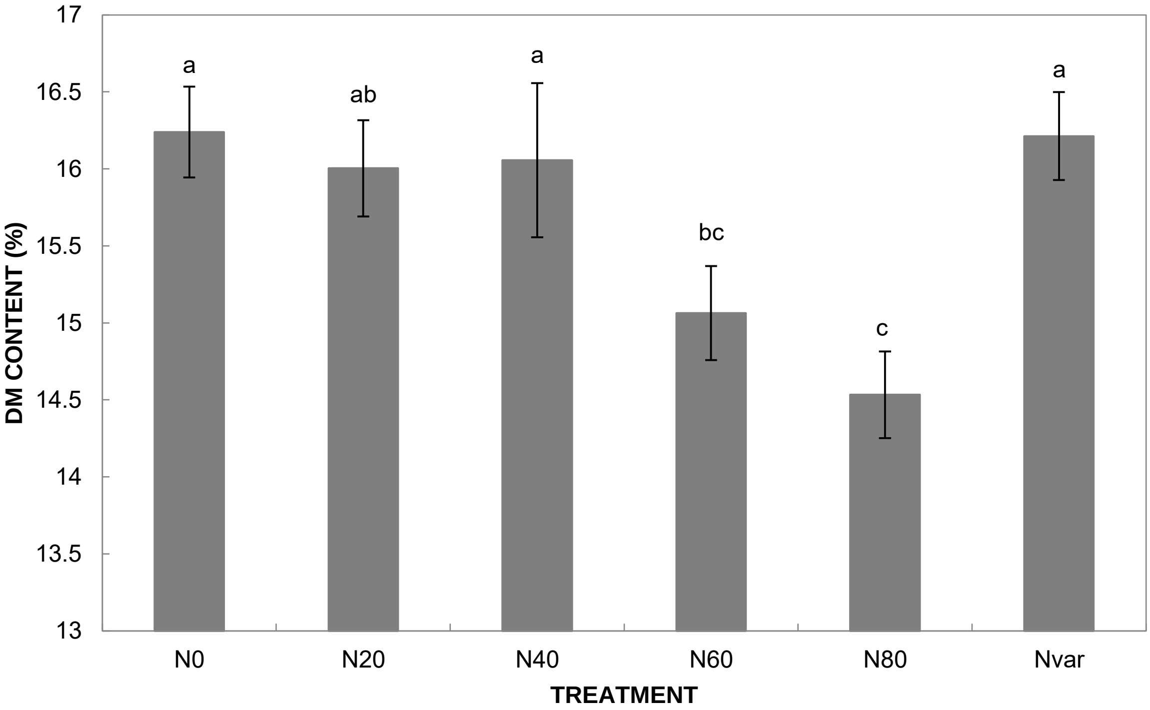

Figure 4.24: Average dry matter (DM) content (%) of the kikuyu-ryegrass site, when averaged over grazing cycles from May 2016 to March 2018 and affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$ and Nvar = varying N rate according to nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

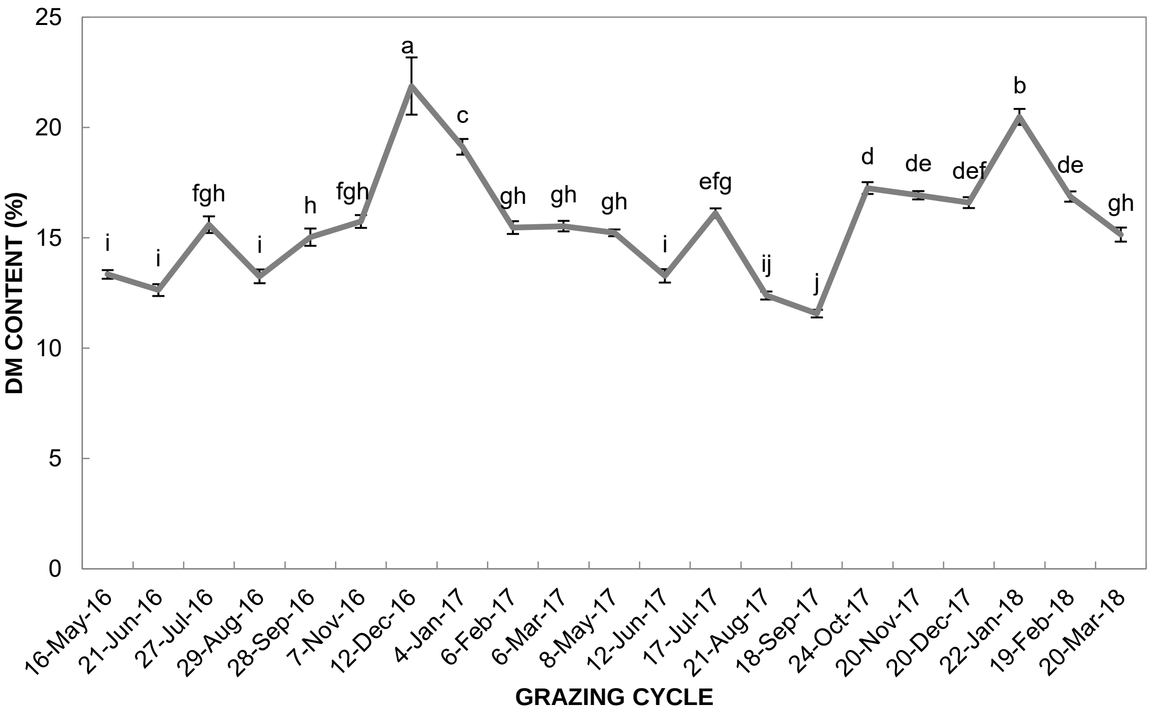

Figure 4.25: Dry matter (DM) content (%) of the kikuyu-ryegrass site, when averaged over treatments and affected by the grazing cycles. Error bars indicate standard error. No common letter above data points indicates significant difference at 5% level

4.3.2.1.5 Dry matter content per season

The response of seasonal dry matter (DM) content were similar (P>0.05) for both season and treatment (Table 4.4). The DM content of treatments was higher (P≤0.05) in spring and summer than compared to winter and autumn of year one. Summer of year two had higher (P≤0.05) DM contents compared to winter and spring. This was similar to what van der Colf et al. (2015a) found, in that summer DM content of kikuyu-ryegrass was higher compared to winter, spring and autumn during year one of the study. During year two, they found that summer and autumn had higher DM content compared to winter and spring. On the kikuyu-ryegrass site during this study, there were no treatment effects (P>0.05) during autumn. One of the reasons for this might be that when the kikuyu base was over-sown with ryegrass for re-establishment, no N was applied to prevent a competitive advantage from the kikuyu base. Another reason might be that there was only one N application during autumn (month of May) while the other seasons received approximately three applications. The pasture was over-sown in March 2017 with no N application, and then grew for two months before it was ready to be grazed again in May 2017 (after which N application commenced).

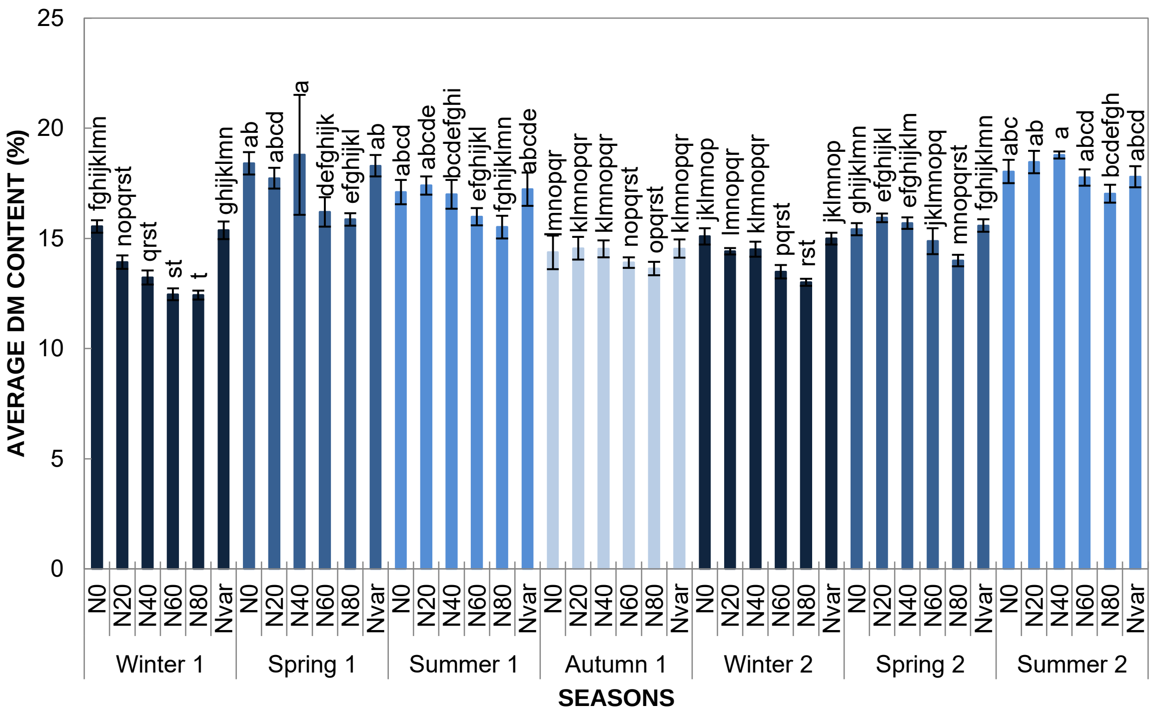

Figure 4.26: Average seasonal dry matter (DM) content (%) of the kikuyu-ryegrass site in seasons during year one (1) and two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60 and 80 kg N ha^{-1} and Nvar = varying rate of N. N Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level.

In winter, spring and summer of year one and winter of year two, treatment N0 had a higher ($P \leq 0.05$) DM content compared to N80 (Figure 4.26). During both winters and spring and summer of year one, Nvar also had a higher ($P \leq 0.05$) in DM content than N80.

On the same site, a previous study found mean DM content to be 13.1, 12.8, 15.5 and 12.2% during winter, spring, summer and autumn of year one, respectively, and 13.2, 12.1, 16.2 and 16.8% in winter, spring, summer and autumn of year two respectively (van der Colf 2011). The mean DM content of the current study was consistently higher, compared to the previously mentioned study, viz.13.8% (winter 1), 17.6% (spring 1), 16.7% (summer 1), 14.3% (autumn 1), 14.3% (winter 2), 15.3% (spring 2) and 18.0% (summer 2).

4.3.2.2 Botanical composition

4.3.2.2.1.1 Kikuyu

Kikuyu's contribution response to the treatments was similar ($P > 0.05$) in all seasons (Table 4.4). Season, however, did have an effect ($P \leq 0.05$) (Figure 4.27). During winter of year one the kikuyu content of treatment N0 was higher ($P \leq 0.05$) than the rest. During spring and summer of year one and winter and spring of year two, there were no treatment differences ($P > 0.05$). In autumn of year one, N80 resulted in higher ($P \leq 0.05$) kikuyu contribution compared to all other treatments, except N60 which had similar ($P > 0.05$) contribution to N80. During summer of year two, N40 contributed more ($P \leq 0.05$) kikuyu than N0, while during autumn the N20 treatment had a higher ($P \leq 0.05$) kikuyu contribution than when compared to N0 (Figure 4.27). On the similar location as the current study, contribution of kikuyu on a kikuyu-Italian ryegrass pasture was 10.7% during winter, 1.3% (year 1) and 3.5% (year 2) during spring, 43.3% (year 1) and 44.8% (year 2) during summer and 26.7% (year 1) and 95.1% (year 2) during autumn (van der Colf et al. 2015a). These results were comparable with the mean kikuyu contributions of the current study at 13.1%, 7.6%, 35.3% and 50.2% during year one, and 2.4%, 1.0%, 48.5% and 64.6% during year two for winter, spring, summer and autumn, respectively.

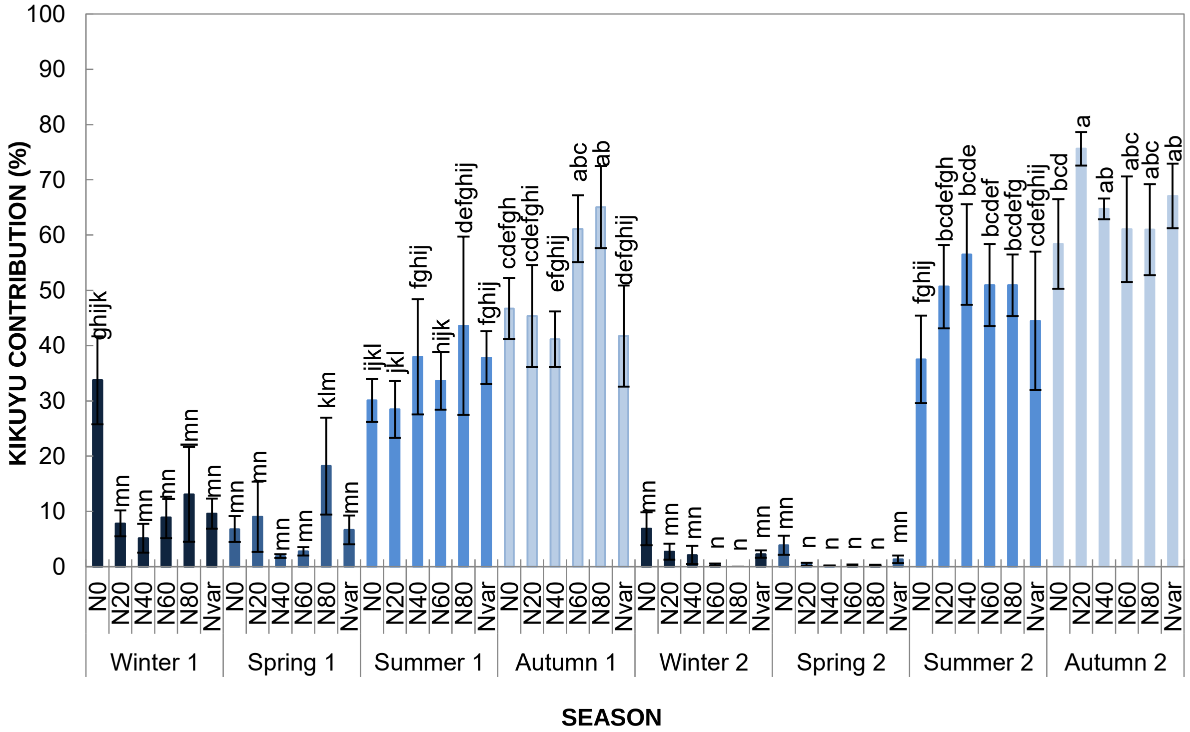

Figure 4.27: Kikuyu contribution (%) to the kikuyu-ryegrass site botanical composition in seasons during year one (1) and year two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

4.3.2.2.1.2 Ryegrass

The response of ryegrass contribution did not vary (P>0.05) according to treatment and season interactions (Table 4.4). The ryegrass was, however affected (P≤0.05) by treatment and season as main effects. The seasons in which ryegrass contributed the most (P≤0.05), within treatments, was winter and spring of both years (Figure 4.28). During winter, spring and autumn of year one, treatment N40 ryegrass contributed more (P≤0.05) to the pasture than in the N0 treatment. There were no treatment differences (P>0.05) during summer of the first year. During winter of year two both treatment N20 and N80 the contribution of ryegrass to the pasture was greater (P≤0.05) than for N0. Treatment N40 resulted in higher (P≤0.05) ryegrass contribution compared to N0 but similar (P>0.05) to N20, N60 and N80 during spring of the second year. Treatments did not affect (P>0.05) the amount of ryegrass in the pasture during summer and spring of the second year. In New Zealand N applications of 100 kg N ha^{-1} in spring and autumn, resulted in an increase in the ryegrass component and a decrease in the clover component (Sun et al. 2008). At the same location as the current study, the contribution of Italian ryegrass on a kikuyu-Italian ryegrass pasture was 80.3% during winter, 93.5% (year 1) and 93.2% (year 2) during spring, 42.9% (year 1) and 39.8% (year 2) during summer and 43.8% (year 1) and 1.9% (year 2) during autumn (van der Colf et al. 2015a). The mean ryegrass contributions of the current study was 60.6%, 75.4%, 30.6% and 32.7% during the first year and 83.4%, 76.9%, 19.7% and 15.3% during year two of seasons winter, spring, summer and autumn respectively. The results of the contribution of both years of this trial was lower than that found by van der Colf et al. (2015a).

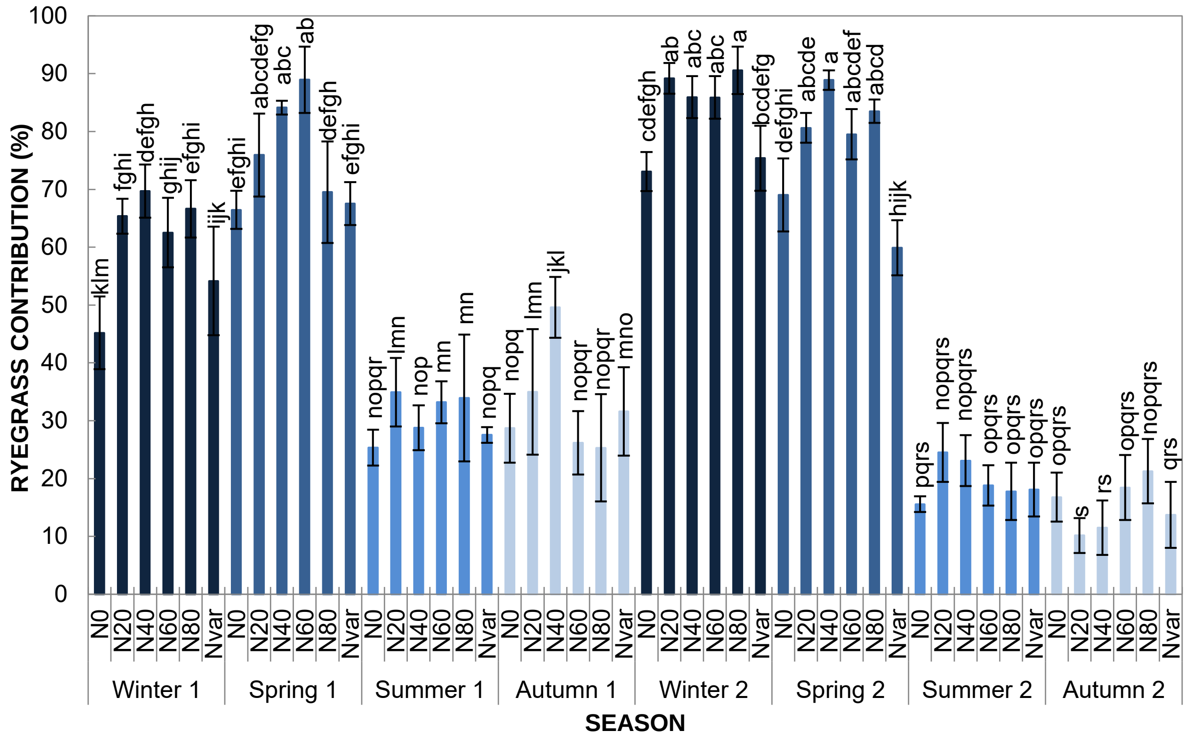

Figure 4.28: Ryegrass contribution (%) to the kikuyu-ryegrass site botanical composition in seasons during year one (1) and year two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

4.3.2.2.1.3 Volunteer legumes

The response of volunteer legumes varied (P≤0.05) according to season and treatment (Table 4.4). The volunteer legumes consisted mostly of white clover and are referred to as "volunteer" because it was not established into the pasture. During winter of year one and autumn of year two, there were no treatment differences (P>0.05) (Figure 4.29). In autumn of year one N0, N20 and Nvar had a higher (P≤0.05) legume contribution compared to N40, N60 and N80. During winter of year two, Nvar contributed more (P≤0.05) legumes to the pasture compared to the other treatments, with the exception of N0 which was similar (P>0.05). During spring of year one, Nvar contributed lower (P≤0.05) legumes compared to N0, but similar (P>0.05) to the rest. Spring of year two resulted in Nvar contributing most (P≤0.05) to the pasture compared to the other treatments. A review article (Sun et al. 2008) stated that the competitive ability of clovers is reduced in cool seasons compared to warmer seasons and that clover contribution increased with decreasing N rate (Elliott and Abbott 2003). This was also evident in the current study.

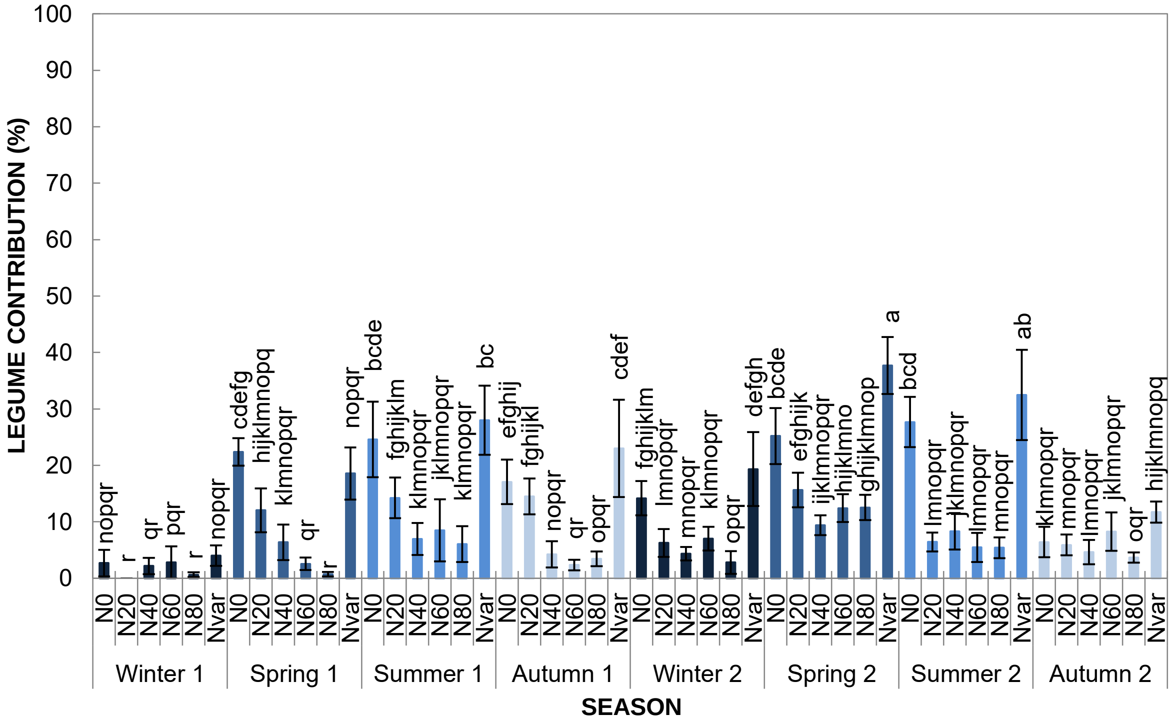

Figure 4.29: Volunteer legume contribution (%) to the kikuyu-ryegrass site botanical composition in seasons during year one (1) and year two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$ and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

4.3.2.2.1.4 Non-metric multidimensional scaling

The non-metric multidimensional scaling (NMDS) ordination fit is sufficient (stress below 0.2) in two dimensions, resulting in only one graph with a stress of 0.14 (Figure 4.30). The correlation between N application and the ordination was significant (P=0.001) with an R^2=0.21.

Axis one displays the shift in botanical composition from predominately kikuyu, weeds and other grasses during summer and autumn, to ryegrass dominated pasture in winter and spring. It is also possible to deduce that composition varied between year one and year two, since the open and shaded shapes are not clustered together. According to the data ryegrass is expected to increase with an increase in N application; however the correlation is not strong (short arrow). Figure 4.28 support this, where treatments had an effect in some seasons (winter, spring, autumn of year one and winter and spring of year two) but not in others. The almost negative correlation that exists between kikuyu and N application is explained by Figure 4.27. Here it is seen that kikuyu does not keep increasing with an increasing amount of N, but that the highest contribution is often not associated with high amount of applied N. Volunteer legumes and N application rate were negatively correlated, resulting in a higher legume contribution with lower N applications.

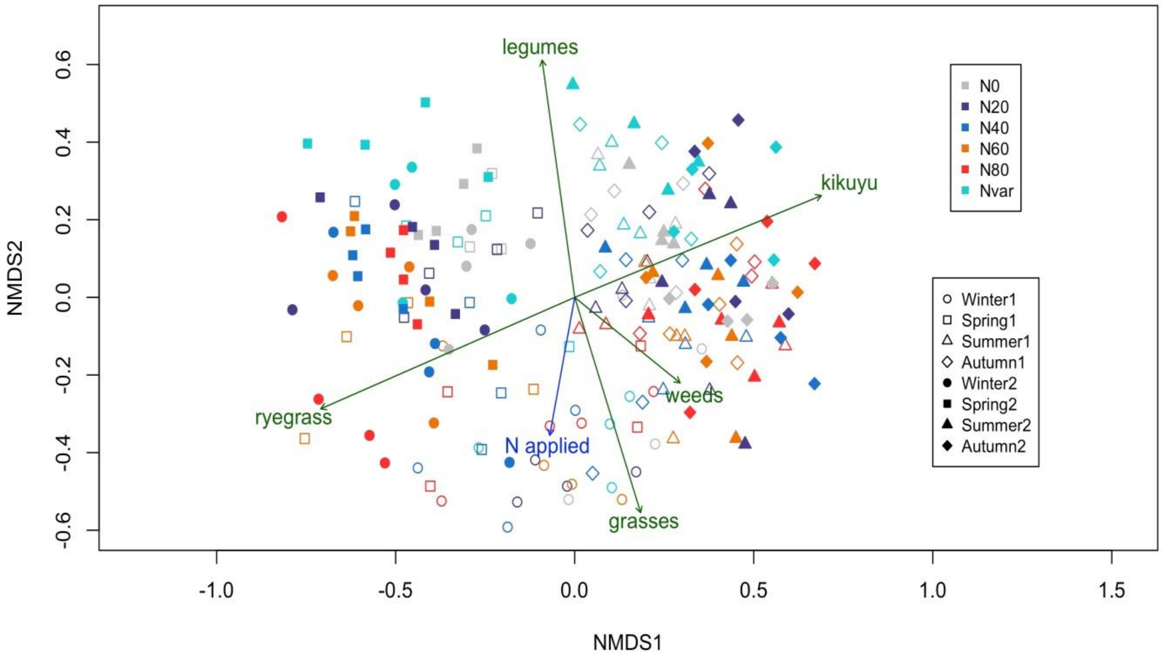

Figure 4.30: Nonmetric multidimensional scaling (NMDS) ordination, axis 1 and 2 of botanical composition components (kikuyu, ryegrass, volunteer legumes, other grasses and weeds) in kikuyu-ryegrass site as influenced by season (winter, spring, summer and autumn of year one and two) and treatments (N0, N20, N40, N60, N80 =0, 20, 40, 60, 80 kg N ha^{-1} and Nvar = varying N rate according nitrate concentration in the soil water

4.3.2.2.2 Agronomic N use efficiency

The response of seasonal agronomic N use efficiency (ANUE) was similar (P>0.05) to treatments in all seasons (Table 4.4). During winter of year one, treatment N20 had a higher (P≤0.05) ANUE compared to N60 and N80. After that, there were no differences (P>0.05) between the treatments in the various seasons (Figure 4.31). In a previous study ANUE was found to be unaffected by treatment and varied greatly between years and location of experiments (Lowe et al. 2005), with it being lowest in winter and autumn, and highest in spring (Elliott and Abbott 2003; Bolland and Guthridge 2007). There was generally an inverse relationship between ANUE and N application rate, but difference between treatments were also not significant in this study (Lowe et al. 2005). Marino et al. (2004) found significant inverse relationships between N application rate and ANUE (Miles 1991; Garcia et al. 2008; Fessehazion et al. 2011). Fessehazion et al. (2011) found the highest NUE was found when 20 kg N ha^{-1} were applied and the lowest ANUE was seen at 60 kg N ha^{-1} rates. The mean NUE reported by Fessehazion et al. (2011) was approximately 30 kg DM kg^{-1} N ha^{-1} and validated by other authors (Garcia et al. 2008). In a different study the range of NUE was between 0 and 62 kg DM kg^{-1} N ha^{-1} (Bolland and Guthridge 2007). According to Theron and van Rensburg (1998), as the defoliation interval increased from two weeks to eight weeks on ryegrass, the ANUE increased, but within each defoliation treatment high N applications (450 kg N ha^{-1} after defoliation) resulted in lowered ANUE.

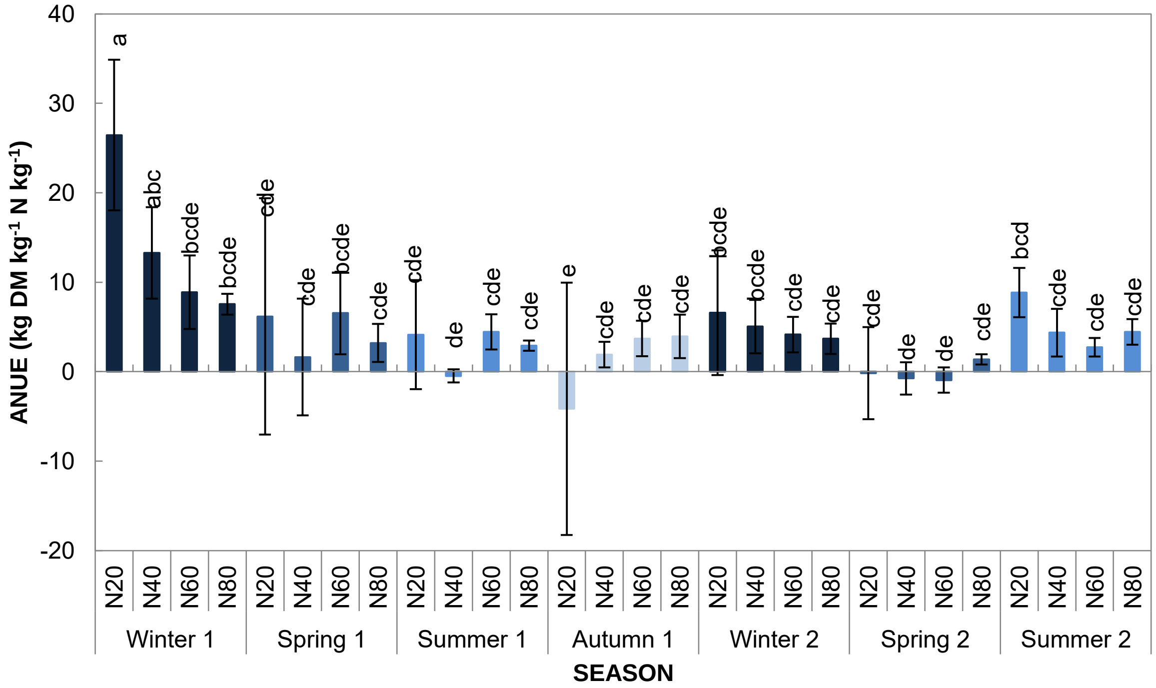

Figure 4.31: Agronomic nitrogen use efficiency (ANUE) (kg DM kg^{-1} N ha^{-1}) of the kikuyu-ryegrass site in seasons of year one (1) and two (2) as affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing cycle^{-1} and Nvar = varying N rate according nitrate concentration in the soil water.. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

4.3.2.2.3 Crude protein

There was an interaction (P≤0.05) between treatments and the average crude protein (CP) content over season (Table 4.4). Within all seasons, treatment N80 was higher (P≤0.05) compared to N0, N20 and Nvar (Figure 4.32). The CP of N20 and Nvar remained similar (P>0.05) to one another when compared within seasons, throughout year one (Figure 4.32). Winter during both years of the study had a higher (P≤0.05) CP compared to both spring and summer of year one. Botha et al. (2008a) reported in a study at the same location, regarding ryegrass sown into kikuyu, spring CP (during year two and three of the study) was higher compared to autumn and summer. This was not found in the current study where spring and summer were the lowest (P≤0.05) in CP content.

As N fertilisation increased, N concentration increased in N foliage, while the source of N had little effect on N content (Lowe et al. 2005). This is similar to what was found in the current study.

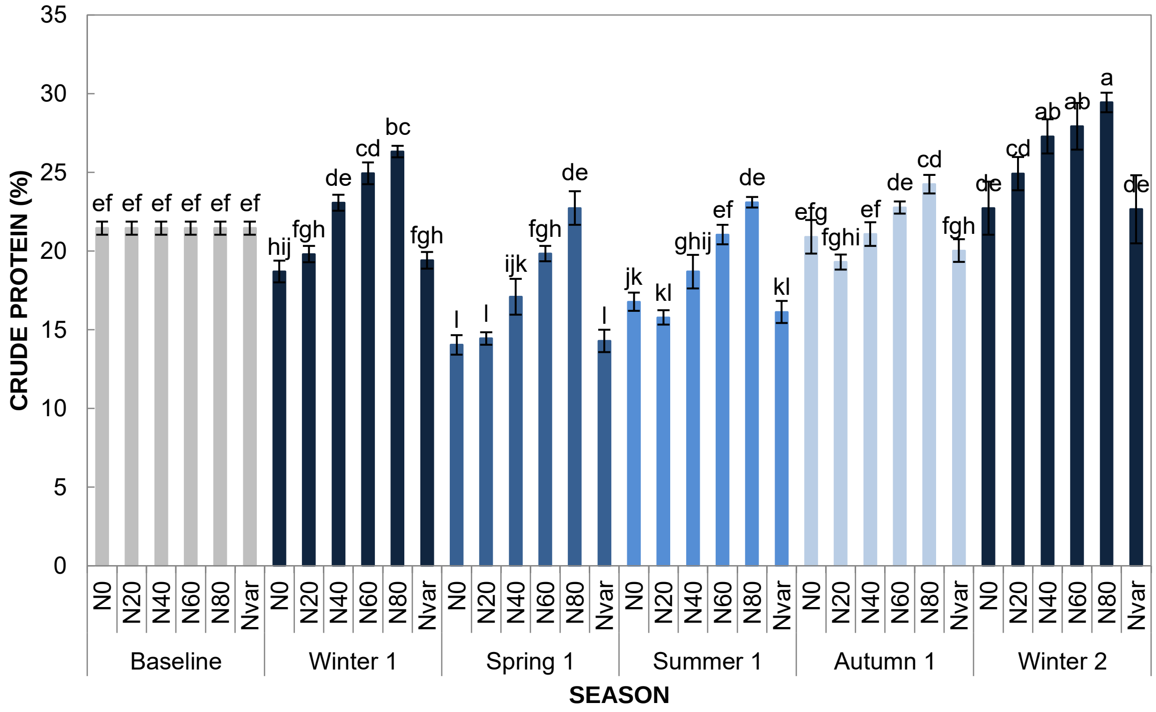

Figure 4.32: Average crude protein (CP) content (%) of the kikuyu-ryegrass site in seasons of year one (1) and two (2) and affected by treatments N0, N20, N40, N60, N80 = 0, 20, 40, 60, 80 kg N ha^{-1} grazing $cycle^{-1}$ and Nvar = varying N rate according nitrate concentration in the soil water. Error bars indicate standard error. No common letter above bars indicates significant difference at 5% level

4.4 Conclusion

On the kikuyu site during winter of year one, the addition of any amount of N between 20 and 80 kg N ha^{-1} $grazing^{-1}$, resulted in an increase in production compared to when no N was applied. When applying 40 kg N ha^{-1} during this season, it resulted in the highest volunteer ryegrass content and lowest kikuyu content compared to the other treatments. In addition it resulted in a CP of 22.73% which is higher than what is required for small breed dairy cows. During winter of year two treatments did not have an effect on the DM production, but a similar trend was seen in the botanical composition regarding a higher volunteer ryegrass content and lower kikuyu content in the N40 treatment. During spring, the highest production was found in the N40 treatment, though it was not significantly higher than the other treatments. In addition, the pasture for this treatment consisted primarily of volunteer ryegrass (>47%) and had a CP content of 16.8%. In summer, applying N in the range of 20 to 80 kg N ha^{-1} resulted in similar production with a kikuyu contribution to the pasture of more than 48%. All treatments below 60 kg N ha^{-1} resulted in a CP content of 18% or less. In autumn, 60 kg N ha^{-1} after every grazing resulted in the highest DM production.

On the kikuyu-ryegrass site, during winter of year one, applying any amount of N resulted in a production higher than when applying no N. In addition, any rate of N fertilisation resulted in a higher ryegrass content compared to no N. The CP, when applying 20 kg N ha^{-1}, resulted in the best treatment in terms of forage quality during this season. During spring, N application rates as low as 20 kg N ha^{-1} after every grazing will result in similar yields than when applying N80. It also resulted in a more favourable CP content of 14.44%. This was true for summer, autumn of year one and winter of year two. Due to the variability in results likely due to the impacts of existing N reserves in the soil, it may be warranted to undertake longer term studies at these rates.

4.5 References

AOAC. 2000. Official method of analysis 988.05 (17th edn) Volume I. Association of Official Analytical Chemists, Inc., Maryland, USA.

Baligar VC, Fageria NK, He ZL. 2001. Nutrient Use Efficiency in Plants. *Communications in Soil Science and Plant Analysis* 32: 921–950.

Barth Neto A, Savian JV, Tres Schons RM, Bonnet OJF, Canto MW do, Moraes A de, Lemaire G, de Faccio Carvalho PC. 2014. Italian ryegrass establishment by self-seeding in integrated crop-livestock systems: Effects of grazing management and crop rotation strategies. *European Journal of Agronomy* 57: 77–83.

Bester C. 2014. Nitrogen management strategies for mixed pastures grown under irrigation in the Winelands sub-region of the Western Cape. MSc(Agric) thesis, University of Stellenbosch, Stellenbosch, South Africa.

Beyers C de L. 1994. Die bemesting van aangeplante weidings. *Weidings = Pastures Elsenburg: Department of Agriculture*: 86–93.

Bolan NS, Horne DJ, Currie LD. 2004. Growth and chemical composition of legume-based pasture irrigated with dairy farm effluent. *New Zealand Journal of Agricultural Research* 47: 85–93.

Bolland MDA, Guthridge IF. 2007. Responses of intensively grazed dairy pastures to applications of fertiliser nitrogen in south-western Australia. *Australian Journal of Experimental Agriculture* 47: 927–941.

Botha PR. 2003. Die produksiepotensiaal van oorgesaaide kikoejoeweiding in die gematigde kusgebied van die Suid-Kaap. PhD thesis, University of the Freestate, Bloemfontein, South Africa.

Botha PR, Meeske R, Snyman HA. 2008a. Kikuyu over-sown with ryegrass and clover: dry matter production, botanical composition and nutritional value. *African Journal of Range and Forage Science* 25: 93–101.

Botha PR, Meeske R, Snyman HA. 2008b. Kikuyu over-sown with ryegrass and clover: grazing capacity, milk production and milk composition. *African Journal of Range and Forage Science* 25: 103–110.

Canfield DE, Glazer AN, Falkowski PG. 2010. The Evolution and Future of Earth's Nitrogen Cycle. *Science* 330, 192–196.

Cassman KG, Dobermann AR, Walters DT. 2002. Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management. *Ambio* 31: 132–140.

Clark DA, Harris SL. 1996. White clover or nitrogen fertiliser for dairying? *Special Publication-Agronomy Society of New Zealand* 6: 107–114.

Cook BG, Mulder JC. 1984. Responses of nine tropical grasses to nitrogen fertilizer under rain-grown conditions in south-eastern Queensland 1. Seasonal dry matter productivity. *Australian Journal of Experimental Agriculture and Animal Husbandry* 24: 410–414.

Cross GW. 1979. The production potential of *Pennisetum clandestinum* in the midlands and on the south coast of Natal. *Proceedings of the Annual Congresses of the Grassland Society of Southern Africa* 14: 53–59.

Dugmore TJ. 2011. The Effect of Nitrogen Fertilization and Stage of Re-growth on the Nutritive Value of Kikuyu in the Midlands. PhD thesis, University of KwaZulu Natal, Pietermaritzburg, South Africa.

Dugmore TJ, du Toit JH. 1988. The chemical composition and nutritive value of kikuyu pasture. *South African Journal of Animal Science* 18: 72–75.

Eckard RJ. 1989. The response of Italian ryegrass to applied nitrogen in the Natal Midlands. *Journal of the grassland society of Southern Africa* 6: 19–22.

Eckard RJ, Bartholomew PE, Tainton NM. 1995. The yield response of annual ryegrass (*Lolium multiflorum*) to varying nitrogen fertilizer application strategies. South African *Journal of Plant and Soil* 12: 112–116.

Eckard RJ, Franks DR. 1998. Strategic nitrogen fertiliser use on perennial ryegrass and white clover pasture in north-western Tasmania. *Australian Journal of Experimental Agriculture* 38: 155–160.

Elliott DE, Abbott RJ. 2003. Nitrogen fertiliser use on rain-fed pasture in the Mt Lofty Ranges, South Australia. 1. Pasture mass, composition and nutritive characteristics. *Australian Journal of Experimental Agriculture* 43: 553–577.

Fessehazion MK, Stirzaker RJ, Annandale JG, Everson CS. 2011. Improving nitrogen and irrigation water use efficiency through adaptive management: A case study using annual ryegrass. *Agriculture, Ecosystems and Environment* 141: 350–358.

Fourie I. 2015. Production potential of lucerne (*Medicago sativa*) over-sown into kikuyu (*Pennisetum clandestinum*) based pasture. MSc thesis, Nelson Mandela Metropolitan University, George, South Africa.

Fulkerson WJ, Neal JS, Clark CF, Horadagoda A, Nandra KS, Barchia I. 2007. Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Grasses and legumes. *Livestock Science* 107: 253–264.

Fulkerson WJ, Slack K, Havilah E. 1999. The effect of defoliation interval and height on growth and herbage quality of kikuyu grass (*Pennisetum clandestinum*). *Tropical Grasslands* 33: 138–145.

Fulkerson WJ, Slack K, Hennessy DW, Hough GM. 1998. Nutrients in ryegrass, white cloveer and kikuyu pastures in relation to season and stage of regrowth in a subtropical environment. *Australian Journal of Experimental Agriculture* 38: 277–240.

Garcia SC, Fulkerson WJ, Brookes SU. 2008. Dry matter production, nutritive value and efficiency of nutrient utilization of a complementary forage rotation compared to a grass pasture system. *Grass and Forage Science* 63: 284–300.

Garcia SC, Islam MR, Clark CEF, Martin PM. 2014. Kikuyu-based pasture for dairy production : a review. *Crop and Pasture Science* 65: 787–797.

Glass GV, Peckham PD, Sanders JR. 1972. Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. *Review of Educational Research* 42: 237-288.

Goold GJ. 1979. Effect of nitrogen and cutting interval on production of grass species swards in Northland, New Zealand. *New Zealand Journal of Experimental Agriculture* 7: 353–359.

Harris DI, Bartholomew PE. 1991. The production of four ryegrass cultivars oversown at various seeding rates into irrigated kikuyu. *Journal of the Grassland Society of Southern Africa* 8: 82–85.

Harris SL, Clark DA, Waugh CD, Clarkson FH. 1996. Nitrogen fertiliser effects on white clover in dairy pastures. *Special Publication-Agronomy Society of New Zealand* 11: 119–124.

Høgh-Jensen H, Schjoerring JK. 1997. Interactions between white clover and ryegrass under contrasting nitrogen availability : N_2 fixation , N fertilizer recovery , N transfer and water use efficiency. *Plant and Soil* 197: 187–199.

ISTA 2016. International Rules for Seed Testing. 2016: i-284.

Kenney PA, Black JL, Colebrook WF. 1984. Factors Affecting Diet Selection by Sheep. III Dry Matter Content and Particle Length of Forage. *Australian Journal of Agricultural Research* 35: 831–838.

King C, McEniry J, Richardson M, O'Kiely P. 2012. Yield and chemical composition of five common grassland species in response to nitrogen fertiliser application and phenological growth stage. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science* 62: 644–658.

Labuschagne J, Agenbag GA. 2006. The effect of fertiliser N rates on growth of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) grown at high soil water levels under controlled conditions. *South African Journal of Plant and Soil* 23: 215–224.

Ledgard SF, Penno JW, Sprosen MS. 1999. Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. *Journal of Agricultural Science* 132: 215–225.

Lowe KF, Fulkerson WJ, Walker RG, Armour JD, Bowdler TM, Slack K, Knight RI, Moody PW, Pepper PM. 2005. Comparative productivity of irrigated short-term ryegrass (*Lolium multiflorum*) pasture receiving nitrogen, grown alone or in a mixture with white (*Trifolium repens*) and Persian (*T. resupinatum*) clovers. *Australian Journal of Experimental Agriculture* 45: 21–39.

Lowe KF, Hume DE, Fulkerson WJ. 2011. Forages and Pastures | Perennial Forage and Pasture Crops – Species and Varieties. In: Fuquay JW, Fox PF, McSweeney PLH (eds), *Encyclopedia of Dairy Science* (2nd edn). Academic Press 576–585.

Marais JP. 1980. Effect of nitrate and non-protein organic nitrogen levels in Kikuyu (*Pennisetum clandestinum*) pastures on digestibility in vitro. *Agroanimalia* 12: 7–11.

Marais JP. 1990. Relationship between nitrogen and other chemical components in kikuyu grass from long-established pastures. *South African Journal of Animal Science* 20: 147–151.

Marais JP. 2001. Factors affecting the nutritive value of kikuyu grass. *Tropical Grasslands* 35: 65–84.

Marais JP, Figenschou DL, Woodley GAJ. 1990. Energy deficiency in kikuyu grass containing high levels of nitrogen. *South African Journal of Animal Science* 20: 16-20.

Marino MA, Mazzanti A, Assuero SG, Gastal F, Echeverría HE, Andrade F. 2004. Nitrogen dilution curves and nitrogen use efficiency during winter-spring growth of annual ryegrass. *Agronomy Journal* 96: 601–607.

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A. 2010. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Annals of Botany* 105: 1141–1157.

Meeske R, Rothauge A, van der Merwe GD, Greyling JF. 2006. The effect of concentrate supplementation on the productivity of grazing Jersey cows on a pasture based system. *South African Journal of Animal Sciences* 36: 105-110.

Miles N. 1991. Nutrition of intensive pastures in the summer rainfall areas of southern Africa. *Journal of the Grassland Society of Southern Africa* 8: 39–48.

Miles N. 1997. Responses of productive and unproductive kikuyu pastures to top - dressed nitrogen and phosphorus fertiliser. *African Journal of Range and Forage Science* 14: 1–6.

Miles N, Thurtell L, Riekert S. 2000. Quality of Kikuyu herbage from pastures in the Eastern Cape coastal belt of South Africa. *South African Journal of Animal Science* 30: 85–86.

Nevens F, Rehuel D. 2003. Effects of cutting,grazing grass swards on herbage yield, N uptake and residual soil N at different levels of N fertilization. *Grass and Forage Science* 58: 431–449.

NRC (National Research Council). 2001. *Nutrient requirements of dairy cattle*. National Academy Press: Washington, DC.

Pearson CJ, Kempb I, Kirbyc AC, Laundersd TE, Mikled C. 1985. Responsiveness to seasonal temperature and nitrogen among genotypes of kikuyu , paspalum and bermuda grass pastures of coastal New South Wales. *Australian Journal of Experimental Agriculture* 25: 109–116.

Pembleton KG, Rawnsley RP, Burkitt LL. 2013. Environmental influences on optimum nitrogen fertiliser rates for temperate dairy pastures. *European Journal of Agronomy* 45: 132–141.

Peoples MB, Baldock JA. 2001. Nitrogen dynamics of pastures: nitrogen fixation inputs, the impact of legumes on soil nitrogen fertility, and the contributions of fixed nitrogen to Australian farming systems. *Australian Journal of Experimental Agriculture* 41: 327–346.

Reeves M, Fulkerson WJJ, Kellaway RC. 1996. Forage quality of kikuyu (*Pennisetum clandestinum*): The effect of time of defoliation and nitrogen fertiliser application and in comparison with perennial ryegrass (*Lolium perenne*). *Australian Journal of Agricultural Research* 47: 1349–1359.

Tamimi YN, Sherrod LB, Ishizaki SM, Izuno T. 1968. The Effect of Levels of Nitrogen, Phosphorus, and Potassium Fertilization upon Beef Production on Kikuyugrass. Hawaii Agricultural Experiment Station, University Of Hawaii. *Technical Bulletin* No. 76

Sinclair K, Beale PJ. 2010. Critical factors influencing no-till establishment of short-term ryegrass (*Lolium multiflorum*) into a kikuyu (*Pennisetum clandestinum*) pasture. *Crop and Pasture Science* 61: 192–200.

Snedecor GW, Cochran WG. 1980. Statistical methods, 7th edn. Ames, Iowa State University Press, pp 507.

Stirzaker RJ. 2003. When to turn the water off: Scheduling micro-irrigation with a wetting front detector. *Irrigation Science* 22: 177–185.

Sun X, Luo N, Longhurst B, Luo J. 2008. Fertiliser Nitrogen and Factors Affecting Pasture Responses. *The Open Agriculture Journal* 2: 35–42.

Swanepoel PA, Botha PR, Snyman HA, Preez CC. 2014. Impact of cultivation method on productivity and botanical composition of a kikuyu-ryegrass pasture. *African Journal of Range and Forage Science* 31: 215–220.

Syvertsen JP, Smith ML. 1996. Nitrogen Uptake Efficiency and Leaching Losses from Lysimeter-grown Citrus Trees Fertilized at Three Nitrogen Rates. *Journal of the American Society for Horticultural Science* 121: 57–62.

Theron JF, van Rensburg WLJ. 1998. The influence of nitrogen and defoliation on production and water-use efficiency of *Lolium multiflorum* cv. Midmar. *African Journal of Range and Forage Science* 15: 35–40.

TIBCO Software. 2017. Statistica (data analysis software system), version 13. TIBCO Softw., Palo Alto, CA.

van der Colf J. 2011. The production potential of Kikuyu (Pennisetum clandestinum) pastures over-sown with Ryegrass (Lolium spp.). MSc(Agric) thesis, University of Pretoria, Pretoria, South Africa.

van der Colf J. 2016. The evaluation of annual ryegrass varieties in the southern Cape: 2014 to 2015. In 'Inligtingsdag | Informationday', 15–20

van der Colf J, Botha PR, Meeske R, Truter WF. 2015a. Seasonal dry matter production, botanical composition and forage quality of kikuyu over-sown with annual or perennial ryegrass. *African Journal of Range and Forage Science* 32: 133–142.

van der Colf J, Botha PR, Meeske R, Truter WF. 2015b. Grazing capacity, milk production and milk composition of kikuyu over-sown with annual or perennial ryegrass. *African Journal of Range and Forage Science* 32: 143–151.

Venuto BC, Redfearn DD, Pitman WD, Alison MW. 2004. Impact of seeding rate on annual ryegrass performance. *Grass and Forage Science* 59: 8–14.

Vogeler I, Cichota R. 2016. Determination of nitrogen fertiliser requirements in dairy production systems based on early indicators. In: *Efficient use of different sources of nitrogen in* *agriculture–from theory to practice Skara, Sweden 27 June–29 June 2016: 20.*

Ward GNA, Clark SGB, Kearney GAC, Mccaskill MRB, Raeside MCB, Lawson ARD. 2013. Summer-active perennials in pasture systems improve seasonal pasture distribution without compromising winter-spring production. *Crop and Pasture Science* 64: 673–686.

Chapter 5 Summary and recommendations

5.1 Synopsis

Kikuyu over-sown with ryegrass is a common pasture used for dairy production in the southern Cape of South Africa. There is an abundance of literature showing that the pasture production of kikuyu and ryegrass increases with an increase in inorganic nitrogen (N) fertiliser application rate (Le Roux et al. 1984; Eckard 1989; Marais 1990; Eckard et al. 1995; Bolan et al. 2004; Garcia et al. 2014), which in turn allows it to support a high stocking rate. The guidelines which are used for kikuyu-ryegrass systems recommend an N application rate of 300 to 500 kg N ha^{-1} yr^{-1} (Eckard et al. 1995; Marais 2001). In the southern Cape, rates between 200 and 600 kg N ha^{-1} yr^{-1} are commonly applied (Miles et al. 2000). This broad range indicates the need to refine these N application rates, with farming efficiency as the primary goal, so as to prevent financial losses and environmental damage through eutrophication of soil and aquatic systems. Some authors have suggested incorporating legumes such as clovers as a potential strategy to allow for a reduced rate of fertiliser application (Peoples and Baldock 2001; Andrews et al. 2007; Ledgard et al. 2009). However, substandard establishment, poor persistence and lower herbage production when legumes are included, favours pure grass pastures. Research regarding optimum N application regimes is lacking on kikuyu-ryegrass pastures in the southern Cape. A study was conducted on Outeniqua Research Farm to determine the optimum rate of N fertilisation of kikuyu and kikuyu-ryegrass pastures, either by a fixed N fertilisation rate or a variable rate according to the demand of the plant in a specific season. The study was divided into two parts, corresponding to two objectives.

Objective 1: To investigate the effects of fertilisation on soil N dynamics, which will aid in optimising N fertilisation of kikuyu and kikuyu-ryegrass pastures. In order to achieve the objective, the effects of different N rates on various soil characteristics such as potentially mineralisable N (PMN), C:N ratio, urease activity (UA) and total soil N are determined in order to better understand the N dynamics in the soil as influenced by grazing, N fertiliser input and season.

The effects of fertilisation treatments on soil parameters were determined on two sites (pure kikuyu and kikuyu over-sown with ryegrass). Total mineral N was the main parameter that showed a response to the N fertilisation. On both sites, total mineral N indicated that the treatments above 40 kg N ha^{-1} grazing cycle^{-1} resulted in a build-up of N through time in the 0 – 100 mm depth when compared to the control treatment (N0). Even in the 200 – 300 mm soil layer, the total mineral N of both sites, increased in certain grazing cycles when the fertilisation rate increased above 40 kg N ha^{-1} grazing cycle^{-1}. This is evidence that N fertilisation above N40 could likely result in losses through leaching. Increased leaching losses due to increased N applications have been reported in previous studies. In a study conducted by Ledgard et al. (1999), leaching losses on a ryegrass-clover pasture was in the range of 20 to 74 kg N ha^{-1} yr^{-1} when no N was applied. These losses increased to ranges of between 100 and 204 kg N ha^{-1} yr^{-1}, as a result of N fertiliser applications of 400 kg N ha^{-1} yr^{-1}. Therefore, it would be advisable to apply as little N as is necessary for optimal pasture growth in order to prevent financial and environmental losses. The optimal rate for pasture production is discussed under objective 2. However, only considering total mineral N, no more than 40 kg N ha^{-1} grazing $cycle^{-1}$ is advised. Even at 40 kg N ha^{-1}, leaching losses will occur. These losses are confirmed by the use of wetting front detectors (WFD), from which a soil water nitrate concentration sample could be retrieved at 150 and 300 mm soil depths. These nitrate concentrations could be viewed a direct measure of leaching and was almost always in excess of the allowed 50 mg L^{-1} determined by the EEC (1991) for both soil depths. This implies that the soil is saturated with nitrate.

When UA was normalised with total mineral N, the results showed on both sites, that the UA was lowest in high N treatments. However this observation was not always significant. During December 2016, the differences in the normalised UA were the lowest ($P \leq 0.05$) in treatments N60 and N80 (high N fertilisation rates) and this could have been due to the average temperature being the highest (20°C) during this month. A laboratory study confirmed higher microbial activity at higher temperatures (25°C) compared to lower temperatures (5°C and 10°C) (Zak et al. 1999).

Potentially mineralisable N did not vary between treatments on either the pure kikuyu site or the kikuyu over-sown with ryegrass site. On the kikuyu site the range of PMN, including all treatments over all seasons, was -77 to 211 kg N ha^{-1} grazing $cycle^{-1}$, while on the kikuyu-ryegrass site, PMN was in the range of -72 to 231 kg N ha^{-1} grazing $cycle^{-1}$. Even though PMN cannot be used to aid in planning a fertilisation programme as PMN was variable through time, it could still be of value. There is a possible savings by not applying N due to the potential of the current soil, with the help of microbes, to supply N up to rates as high as 231 kg ha^{-1}, which is sufficient to sustain pasture growth.

Leco-N (i.e. total soil N) showed no difference between treatments. Leco-N was, however, affected by season. On the pure kikuyu site the Leco-N changed from winter > spring > summer = autumn. On the kikuyu-ryegrass site winter was similar to spring and both higher than summer and autumn, which were also similar to each other. From the results it appears that the Leco-N is linked to the herbage production of the pasture. When herbage production is high, Leco-N is low and *vice versa*.

The conclusion of this objective is that the minimum N has to be applied to avoid losses through leaching. Low rates of N fertilisation should also maintain a nitrate concentration of below 50 mg L^{-1} in the soil water, which is the acceptable concentration in Europe (EEC 1991). It was evident that the measured parameters were mostly influenced by climatic conditions rather than N fertilisation rate, with the exception of total mineral N and UA. It is therefore advisable to use these two parameters, total mineral N and UA, in future studies of N fertilisation.

Objective 2: The objective was to reassess N fertiliser guidelines of kikuyu and kikuyu-ryegrass pastures in the southern Cape under a minimum-tillage regime and grazing, aimed at optimising the quality of pasture, measured by CP, while still maintaining yield. Developing a strategic N fertiliser programme may aid in preventing environmental and financial losses since agronomic N use efficiency (ANUE) is taken into account.

The effect of fertilisation rates on pasture characteristics was determined. Few herbage production differences were found within a season and between treatments. Where differences were detected, it was mostly between N0 and N80. Within all seasons, on both sites, the production of N20 was similar ($P>0.05$) to that of N80. The main production differences were found between seasons, primarily due to the respective growing seasons of the different pasture species. Annual pasture production, on both sites, was lowest ($P\leq0.05$) with low N treatments (N0 and Nvar) compared with the high N treatments (N60 and N80), while both treatment N20 and N40 were similar to the highest production. Treatment affected the individual components of botanical composition on both sites. Kikuyu's contribution did not vary with treatments during winter and spring on both sites. On the kikuyu-ryegrass site, during summer and autumn, an increase in N rate resulted in an increased contribution from kikuyu. On the pure kikuyu site the kikuyu contribution varied more. The ryegrass component seem to have been favoured by treatment N40 on both sites, although it was not always statistically significant. Volunteer legumes on both sites were negatively correlated with N rate.

Agronomic N use efficiency did not show treatment effects, and can be contributing evidence that the soil was N saturated. There was, however, a slight trend that lower N application resulted in a higher efficiency on the kikuyu site, but less so on the kikuyu-ryegrass site.

Crude protein (CP) content of herbage increased with an increase in N application at both sites. Treatments N0, N20 and Nvar were similar within seasons, and winter and autumn had higher CP compared with summer and spring. On the kikuyu site, no N should be applied in winter and autumn in order to have a favourable CP content for animal production of 16 to 19% (NRC 2001; Radostits et al. 2006). During spring, treatments N0, N20, N40 and Nvar were considered to have favourable CP content, while during summer N0, N20 and Nvar was preferred. Crude protein in the kikuyu-ryegrass site was generally lower than that of the kikuyu site, but still followed similar CP content trends with regards to treatments.

All the above mentioned parameters are taken into account to contribute to the overall big picture to optimise farming efficiency and prevent losses, and therefore the minimum requirements to sustain similar to the highest production with a good CP quality is advised. Since ANUE was similar between treatments, and botanical composition was, with the exception of legume contribution, mostly influenced by season rather than treatments, it would be advised to base fertiliser application rates on yield and CP, while taking total soil N into account.

5.2 General conclusion

The conclusion is based on the results of the current two year study. It would therefore only be applicable to producers with similar conditions to the current study; for instance a previous kikuyu-ryegrass pasture with long-term (10 years) fertilisation of approximately 300 kg N ha^{-1} yr^{-1}.

It is suggested that N fertilisation could be adjusted to season on the kikuyu site. An additional grazing cycle could be obtained during autumn on the kikuyu site since there was no over-sowing of ryegrass, therefore three grazing cycles instead of two. It is recommended that 20 kg N ha^{-1} be applied after every grazing during winter, summer and autumn. During spring, it is advised not to apply any N, in order to utilise the contribution of legumes during this season but more importantly to make use of the N mineralised from organic matter. Such a N fertiliser programme equates to an amount of 180 kg N ha^{-1} yr^{-1} for the kikuyu site, an amount much lower than the current guidelines of between 300 and 500 kg N ha^{-1} yr^{-1}.

On the kikuyu-ryegrass site, it is advised that 20 kg N ha^{-1} is applied after every grazing during the winter and spring seasons, while no N should be applied during summer and autumn. Depending on the number of grazing cycles within the winter season months, which can vary between two or three, the annual application would vary between 100 and 120 kg N ha^{-1} yr^{-1} based on these recommendations. At the above mentioned N application rates within seasons, a favourable CP percentage is maintained, without compromising on pasture production.

With this type of fertilisation regime, it would be to increase profitability, on an economical and environmental basis, on the farm with lowered N input while still maintaining DM production rates.

5.3 Limitation and improvements

In an effort to construct guidelines for strategic fertilisation, WFDs were installed at 150 mm and 300 mm depths to determine the soil water nitrate concentration. The intent was to apply N according to nitrate concentrations in the soil water. However, this strategy was not successful, as the nitrate concentration remained well above 75 mg L^{-1} on the kikuyu and kikuyu-ryegrass sites. In addition, this concentration was higher than the 50 mg L^{-1} as allowed for ground water in Europe (EEC, 1991).

Another limitation of using a WFD in a pasture environment is that the soil did not often reach a soil matrix potential of c. -3 kPa at which a soil water sample could be collected. A sample could be collected mostly when there was a rainfall event after irrigation. The use of WFDs was well received in a study by Fessehazion et al. (2011) on a different soil type, but under controlled conditions and without grazing. Adjusting the irrigation to be able to collect a sample from the WFD is not feasible since this will result in over-irrigation and water wastage. An additional problem with the use of WFD is preventing inquisitive cows from damaging it. In the current study, this was done by putting wire cages over the WFD, but still some cows were able to break the cages and get to the WFD. When the WFD is damaged or moved by the cows, the time it takes to settle in the soil is about a month, during which the results are not reliable.

A further limitation of the study was the inability to separate the treatments from one another during grazing in order to allow cows to only graze one treatment at a time. By doing so, it would have been possible to evaluate total mineral N with the certainty that N from excreta were due to the specific treatments the cows grazed. This might have prevented the overestimation of mineral N in N0 plots and underestimation of N80 plots. For instance, N0 plots would have only received urine from cows grazing N0 plots, which might have been lower in N concentration than the current method of study. Such a strategy would also have allowed the authors to obtain the milk production of specific treatments. Cows were able to graze across treatments resulting in N deposition through excreta on all treatments. The assumption, however, is that all excreta were evenly distributed across all treatments and that the carryover effects were similar for all plots.

An improvement to the study would have been to deplete the soil of N prior to the application of treatments in order to better observe treatment effects on soil parameters. N fertilisation was ceased one month prior to onset of the trial, but a longer time was necessary to deplete the soil of N. A large soil residual N might be responsible for the lack of differences between treatments due to long term fertilisation of more than 300 kg N ha^{-1} yr^{-1} on the trial sites.

It might be possible that there was an overestimation of herbage due to the possibility of including herbage from the previous grazing cycle into the next when cutting. It is possible that urine or dung patches occurred and was not grazed, therefore plant material is carried over to the next grazing cycle. Seasonal post-grazing differences occurred, which means that the pasture was likely less palatable during autumn on both sites as a result of fibrous material from kikuyu. This can be overcome by adjusting the harvested herbage sample according the post-grazing disc-meter readings.

5.4 Future research

Future research should continue on the specific study site at the current rates in order to determine the potential effects of long term N deficiency in the control or the effects of long term over-fertilisation in the high N treatments. Methods to determine whether over-fertilisation and leaching occurs, should be investigated since the use of WFD was not successful in the current soil type. These methods should focus on measuring primarily leaching losses.

It is not known if herbage production would be sustained after two years of minimal seasonal fertilisation and therefore research should continue to examine the long term effects of reduced N fertilisation rates.

It would be interesting to estimate the total inputs and outputs of N using a Life Cycle Assessment approach, within a pasture-based dairy farming system. By adding economic aspects into this approach, the efficiency of such a pasture-based dairy farming systems could be determined and assessed accordingly. In addition, climate change is a pertinent topic, with agriculture contributing to a large extent towards greenhouse gas (GHG) emissions, particularly in the form of nitrous oxide. Nitrous oxide are viewed as a greater threat with regards to climate change than carbon dioxide. Policy and consumer pressure to reduce GHG emissions is increasing. As a result thereof, systems and strategies to reduce GHG emissions need to be put in place. Currently there is a lack of information and data about South Africa's GHG inventories and how to accurately predict GHG emissions. There is a need to develop country specific models to predict GHG emissions in these specific agricultural fields. Research focussing on this subject will contribute towards development of programs and set standards by which the South African government can determine possible carbon taxing guidelines within the agricultural sector. However, such a taxing system has not yet been implemented. The current study provides a good background and introduction to estimate and create prediction models of GHG emissions from different N fertilisation application rates. When such a system is implemented, it will be an incentive for pasture-based dairy farmers to apply fertiliser rates more cautiously when carbon taxing becomes a reality in South Africa.

5.5 References

Andrews M, Scholefield D, Abberton MT, Mckenzie BA, Hodge S, Raven JA. 2007. Use of white clover as an alternative to nitrogen fertiliser for dairy pastures in nitrate vulnerable zones in the UK : productivity , environmental impact and economic considerations. *Annals of Applied Biology* 151: 11–23.

Bolan NS, Horne DJ, Currie LD. 2004. Growth and chemical composition of legume-based pasture irrigated with dairy farm effluent. *New Zealand Journal of Agricultural Research* 47: 85–93.

Eckard RJ. 1989. The response of Italian ryegrass to applied nitrogen in the Natal Midlands. *Journal of the grassland society of Southern Africa* 6: 19–22.

Eckard RJ, Bartholomew PE, Tainton NM. 1995. The yield response of annual ryegrass (Lolium multiflorum) to varying nitrogen fertilizer application strategies. *South African Journal of Plant Soil* 12: 112–116.

EEC. 1991 Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. *Official Journal L* 375: 1–8.

Fessehazion MK, Stirzaker RJ, Annandale JG, Everson CS. 2011. Improving nitrogen and irrigation water use efficiency through adaptive management: A case study using annual ryegrass. *Agriculture, Ecosystems and Environment* 141: 350–358.

Garcia SC, Islam MR, Clark CEF, Martin PM. 2014. Kikuyu-based pasture for dairy production : a review. *Crop and Pasture Science* 65: 787–797.

Le Roux CJG, Howe LG, Harrison RA. 1984. The effect of nitrogenous fertilization on nitrogen status of three grasses. *Fertilization society of South Africa* 1: 49–54.

Ledgard SF, Penno JW, Sprosen MS. 1999. Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. *Journal of Agricultural Science* 132: 215–225.

Ledgard S, Schils R, Eriksen J, Luo J. 2009. Environmental impacts of grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research* 48: 209–226.

Marais JP. 1990. Relationship between nitrogen and other chemical components in kikuyu grass from long-established pastures. *South African Journal of Animal Science* 20: 147–151.

Marais JP. 2001. Factors affecting the nutritive value of kikuyu grass. *Tropical Grasslands* 35: 65–84.

Miles N, Thurtell L, Riekert S. 2000. Quality of Kikuyu herbage from pastures in the Eastern Cape coastal belt of South Africa. *South African Journal of Animal Science* 30: 85–86.

NRC (National Research Council). 2001. *Nutrient requirements of dairy cattle*. National Academy Press: Washington, DC.

Peoples MB, Baldock JA. 2001. Nitrogen dynamics of pastures: nitrogen fixation inputs, the impact of legumes on soil nitrogen fertility, and the contributions of fixed nitrogen to Australian farming systems. *Australian Journal of Experimental Agriculture* 41: 327–346.

Radostits OM, Gay CC, Hinchcliff KW, Constable PD. 2006. Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Saunders Ltd.

Zak DR, Holmes WE, MacDonald NW, Pregitzer KS. 1999. Soil Temperature, Matric Potential, and the Kinetics of Microbial Respiration and Nitrogen Mineralization. *Soil Science Society of America Journal* 63: 575.